

Molecular Characterization and Evaluation of Indigenous Microbial Pathways Supporting Phenol Mineralization in Hydrocarbon-Polluted Soils of Gombe, Nigeria

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Abstract

*The contamination of the environment with phenol is a major challenge due to its toxicity, persistence and prevalence in industrially impacted ecosystems. This study investigated the molecular and functional characteristics of six (6) phenol-degrading bacterial isolates (IS, OKA, IVA, EB, EKA, and ONA) which were isolated from hydrocarbon-polluted soils in Gombe metropolis. After the genomic DNA was extracted from each isolate, all of them were subjected to 16S rRNA gene amplification which yielded expected ~1500 bp fragments and confirmed the suitability of the samples for the downstream analyses. The functional gene screening showed the presence of two different phenol-degradation pathways among the isolates: IS, OKA, and IVA had the monooxygenase gene (~1026 bp) which corresponded to their positive indole hydroxylation phenotype, while EB, EKA, and ONA had the multi-component phenol hydroxylase gene (~209 bp). Strain EB, the most efficient of the degraders at an initial phenol concentration of 500 mg/L, was selected to be characterized based on the gene sequencing. Its partial 16S rRNA gene sequence showed 99.8% identity with *Alcaligenes faecalis* and *Alcaligenes phenolicus* whereas phylogenetic reconstruction placed it clearly within the *Alcaligenes* clade. The sequencing of the hydroxylase gene of strain EB further corroborated the close evolutionary and functional relationships with other phenol degrading *Alcaligenes* strains. Together, the results indicated that the soils of Gombe that have been impacted by hydrocarbon have different kinds of indigenous bacteria with specialized capabilities for the mineralization of phenol. The different enzymatic pathways that were monooxygenase and multi-component hydroxylase systems, point to the metabolic adaptability that is present in these bacterial communities. This work emphasizes the strong bioremediation potential of native microbial populations and identifies *Alcaligenes* sp. EB as a promising candidate for future bioaugmentation strategies targeting phenol-contaminated environments.*

Keywords: Hydrocarbon-polluted soils, Monooxygenase, Phenol hydroxylase, *Alcaligenes*

Introduction

Industrial growth has ramped up environmental pollution to levels that our current clean-up methods just can't handle. In Gombe metropolis, human activities like car repairs and transportation using hydrocarbons are the main culprits behind this pollution (Lazarus et al., 2025). Hydrocarbon contamination comes from various sources, including faulty storage systems, vehicle maintenance, fuel transport accidents, and refuelling generators (U.S. Environmental Protection Agency, 2020). This contamination seeps through the soil and into groundwater, raising serious public health issues for those relying on well water. The

environmental impact goes beyond just human health, affecting both land and water ecosystems (Lazarus et al., 2025). Phenolic compounds are significant environmental pollutants released from a range of industrial activities, such as petrochemical plants, auto service centres, paper mills, oil refineries, agrochemical production, and textile manufacturing (Zhang et al., 2011). Industrial wastewater can have phenol concentrations nearing 10 g/L (Basha et al., 2010). Phenol's physical properties, particularly its density, is greater than that of water, which limits how much it can dilute and this leads to on-going toxicity after it's released into the environment. Phenols harm living organisms by damaging proteins and disrupting cell membranes, which can cause cellular harm and affect overall bodily functions (Bodalo et al., 2008). These compounds are highly toxic, persist in the environment, and can accumulate in living organisms, making them a top priority for pollution control (Lazarus et al., 2025). There are several ways people can be exposed to phenol, such as breathing it in, absorbing it through the skin, or drinking contaminated water, all of which can lead to both immediate and long-lasting health issues (Lazarus et al., 2025). On exposure to phenol or its derivatives, you might experience irritation in your skin, eyes, and respiratory system, and in severe cases, it can escalate to neurological issues, heart problems, and even failure of multiple organs (Agency for Toxic Substances and Disease Registry, 2022). If someone is exposed to phenol over a long period, it can lead to liver and kidney damage, birth defects, and even cancer risks, as noted by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2020). The negative effect of phenol in terms of environmental and public health is significant due to its high toxicity, even in small amounts. In Gombe metropolis and nearby areas, the improper disposal of phenol through farming, oil spills, industrial wastewater, and careless disposal of personal care product containers has become a major environmental issue that resonates globally (Ibrahim et al., 2022). In Gombe metropolis, the urban environment faces various contamination challenges from industrial waste, car emissions, vehicle maintenance, and poor waste management practices, all of which allow phenolic compounds to seep into the soil (Lazarus et al., 2025). Previous studies have shown that phenol can accumulate in urban soils due to surface runoff and industrial discharges (Agency for Toxic Substances and Disease Registry, 2024). A quantitative analysis found that phenol concentrations in soil samples from Gombe metropolis ranged from 0.082 to 0.369 mg/ml (Lazarus et al., 2025). This uneven distribution suggests that contamination levels vary across different areas, likely influenced by various environmental factors (Lazarus et al., 2025). The physical and chemical properties of the soil, such as its texture, organic matter content, and moisture levels, play a crucial role in how contaminants are absorbed, retained, and broken down (Knezovich et al., 1988). Point-source pollution from industrial facilities or compromised underground storage infrastructure creates localized contamination hotspots. Groundwater flow patterns facilitate phenol transport through soil profiles, generating spatial concentration gradients across sampling locations (Agency for Toxic Substances and Disease Registry, 2020). Indigenous microbial communities capable of phenol catabolism contribute to differential degradation rates among sites (Basak et al., 2014). Enrichment culture methodology using M9 minimal medium supplemented with phenol as the sole carbon source yielded fourteen (14) bacterial isolates from contaminated soil samples collected at automotive repair facilities throughout Gombe metropolis (Lazarus et al., 2025). Six isolates designated IS, OKA, IVA, EB, EKA, and ONA demonstrated phenol degradation efficiency exceeding 80% within 72 hours of incubation (Lazarus et al., 2025). Enrichment cultivation techniques have been successful in isolating bacteria that can degrade phenol, including species from the genera *Arthrobacter*, *Bacillus cereus*, *Citrobacter*, *Micrococcus*, and *Pseudomonas putida* (Kanekar et al., 1999). The phenotypic and biochemical analysis in the Lazarus et al., (2025) study indicated that isolate OKA is similar to *Enterobacter aerogenes*

or *Klebsiella pneumoniae*, while isolate EB shows traits typical of *Alcaligenes* or *Pseudomonas*. Isolates IVA and EKA seem to be related to *Pseudomonas* or similar groups, whereas IS and ONA exhibit characteristics common to *Staphylococcus* or *Micrococcus* species. The *Pseudomonas* genus is known for its remarkable metabolic flexibility, including the ability to break down complex polymers, making it a dominant player in wastewater treatment systems (Rossello-Mora et al., 1994). Several *Pseudomonas* species capable of degrading phenol have been identified, such as *Pseudomonas cepacia* (Ghadi and Sangodhkar, 1995), *Pseudomonas pictorum* (Chitra et al., 1995), and *Pseudomonas putida* (Loh and Wang, 1998; Kanekar et al., 1999). Recent research has shed light on how *Proteus mirabilis* strains can utilize phenol, although they do so with a lower degradation efficiency compared to other organisms (Usman et al., 2021). Earlier studies have also pointed out that *Alcaligenes* sp, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Staphylococcus equorum* are capable of breaking down phenol (Shebl et al., 2024). Phenol biodegradation by bacteria involves several metabolic pathways, facilitated by specific microorganisms and enzymes. The initial step is the hydroxylation of the phenol ring at the ortho-position, converting phenol into catechol. This reaction is catalyzed by phenol hydroxylase enzymes. Once catechol is formed, it can follow either the ortho-cleavage pathway or the meta-cleavage pathway depending on the specific microorganism (Mei et al., 2019). In the ortho-cleavage pathway, catechol is degraded by the enzyme 1, 2-catechol dioxygenase, producing muconic acid. Muconic acid is subsequently converted into succinate and acetoin, which feed into central metabolic pathways such as the citric acid cycle and the glyoxylate shunt (Long et al., 2016). Alternatively, some bacteria utilize the meta-cleavage pathway, where catechol is broken down by 2, 3-catechol dioxygenase into 2-hydroxy-muconic semialdehyde. This intermediate undergoes several transformations, eventually forming acetaldehyde and pyruvate. These products also integrate into central metabolic pathways, similar to the ortho-cleavage pathway (Long et al., 2016). The choice of pathway depends on the specific microorganism and environmental conditions. Both pathways are critical for phenol biodegradation in the environment and have been extensively studied for their applications in bioremediation of contaminated sites (Hoyos-Hernandez et al., 2014). While both pathways ultimately result in the complete mineralization of phenol into carbon dioxide and water, the ortho-cleavage pathway is considered more efficient due to its higher conversion rate. However, these pathways are not mutually exclusive. Some microorganisms utilize hybrid pathways that incorporate elements of both ortho- and meta-cleavage processes for phenol degradation. These two pathways demonstrate the versatility of bacterial systems in breaking down xenobiotics like phenol, contributing to their role in maintaining environmental health and stability. The enzymes that break down phenol are well understood and show a high degree of substrate specificity, which allows different organisms to efficiently process phenol. Under aerobic conditions, the degradation of phenolic compounds generally starts with a hydroxylation step that transforms phenol into catechol. While catechol retains a toxicity level similar to phenol, it is more readily biodegradable and degrades more rapidly. In bacteria, particularly among *Pseudomonas* species, the meta-cleavage pathway is extensively documented. Both of these reactions are facilitated by either single or multi-component phenol hydroxylase or monooxygenase enzymes, with the hydroxylation stages acting as the rate-limiting steps in the overall degradation process (Shingler et al., 1992; Futamata et al., 2001). Furthermore, genes coding for phenol monooxygenases have been cloned and sequenced from several phenol-degrading microorganisms (Watanabe et al., 2002). These enzymes fall into two categories: single-component and multi-component types, with multi-component phenol hydroxylase (mPH) being the most prevalent in natural settings. Both monooxygenase and dioxygenase systems include forms with either a single or multiple components that share similar structural

characteristics. Typically, multi-component phenol hydroxylases are composed of six subunits, where the largest subunit houses the active catalytic site (Shingler et al., 1992). Nonetheless, some MPH enzymes display differences in substrate specificity when it comes to substituted phenols (Shingler et al., 1992).

This study investigated the molecular and functional characteristics of six (6) phenol-degrading bacterial isolates which were isolated from hydrocarbon-polluted soils in Gombe metropolis. This research bridges several critical gaps in understanding phenol degradation in contaminated environments, especially in relation to indigenous microbial communities in hydrocarbon-polluted soils. It contributes significantly to the knowledge of bacterial diversity, molecular pathways of phenol degradation, and the genetic and functional potential of environmental strains for bioremediation. By focusing on local strains and their specific enzymatic systems, the study lays the groundwork for future applied research in the field of environmental cleanup.

Materials and Methods

Collection and Preparation of Bacterial Cultures

The bacterial isolates used in this study were sourced from previously characterized strains detailed by Lazarus et al. (2025). We retrieved aliquots of isolates labelled IS, OK_A, IV_A, EB, EK_A, and ON_A from glycerol stocks stored at -80°C. Each cryovial was taken out of cryogenic storage, and sterile inoculating loops were used to transfer the frozen culture into nutrient broth for revitalization.

Molecular Identification

For genomic DNA extraction, the Qiagen Genomic Extraction and Purification Kit was used according to the manufacturer's guidelines. 4 mL of nutrient broth containing two-day-old bacterial cells was centrifuged at 14,000 rpm for one minute, and the supernatant was discarded. Next, 200 µL of CTAB buffer was added to the pellet and vortexed for 15 seconds. This was followed by the addition of 500 µL of CTAB along with 7 µL each of proteinase K and β-mercaptoethanol; the mixture was vortexed briefly and incubated at 65°C for at least one hour. Then, 140 µL of the resulting mixture was transferred into a 1.5 mL microcentrifuge tube, and 560 µL of AVL buffer was added. After vortexing for 15 seconds, the sample was left at room temperature for 10 minutes. Following this, 560 µL of absolute ethanol was mixed in using pulse vortexing for 15 seconds and centrifuged briefly at 4,000 rpm for one minute. Next, 630 µL of the solution was transferred into a spin column filter (taking care not to wet the rim) and centrifuged at 8,000 rpm for one minute. The collection tube was then replaced and the remaining solution was processed in the same manner. Subsequently, 500 µL of AW1 buffer was added and centrifuged at 8,000 rpm for one minute, and the column was transferred to a new tube. This was followed by the addition of 500 µL of AW2 buffer and centrifugation at 14,000 rpm for three minutes. To remove any residual buffer, the column was dried by centrifuging it at 14,000 rpm for one minute. Finally, the column was placed in a new 2 mL tube, 60 µL of buffer AVE was added, incubated at room temperature for one minute, and centrifuged at 8,000 rpm for one minute before discarding the column. The extracted DNA was then kept on ice for at least 10 minutes prior to downstream applications and stored at -80°C.

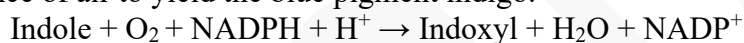
16S rRNA gene Identification

The protocol for detecting the 16S rRNA gene in the bacterial isolates was executed following the method outlined by Weisburg et al. (1991). PCR was utilized to amplify a ~1500 bp fragment of the 16S rRNA gene. Universal primers were employed namely, the forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer 1492R

(5'-GGTTACCTTGTTACGACTT-3'). Each 50 µL PCR reaction contained 1 µg of genomic DNA, with the thermal cycling conditions beginning with an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1.5 minutes, and concluding with a final extension at 72°C for 10 minutes. The successful amplification of the expected PCR products was confirmed via electrophoresis on a 1.5% (wt/vol) agarose gel in TBE buffer, with ethidium bromide used for staining and a λ Hind III digest of lambda DNA serving as the molecular marker.

Identification of Phenol Degrading Gene

The six (6) isolates were further analyzed using PCR to detect relevant degradation genes. Two target genes were investigated: Those encoding monooxygenase and hydroxylase enzymes. An indole hydroxylation test was conducted as described by Prescott et al. (2008). In this reaction, a cytochrome P450 monooxygenase catalyzes the hydroxylation of indole (a typically colourless or pale yellow compound) to form indoxyl, which quickly oxidizes in the presence of air to yield the blue pigment indigo.



Isolates IS, OK_A, and IV_A exhibited a positive indole hydroxylation test, indicating the likely presence of the monooxygenase gene, whereas isolates EB, EK_A, and ON_A, which tested negative, were subsequently targeted for the multi-component phenol hydroxylase gene.

Molecular Protocol for Identification of Monooxygenase Gene

A modified version of the method described by Sambrook et al. (1989) was used to detect the phenol-monooxygenase gene. PCR was performed to amplify a 280 bp fragment using the forward primer 5'-TTGCCAGGTGAAGGTGCTC-3' and the reverse primer 5'-TCTTCTCCGACGCTTCTTCCTGG-3'. The reaction mixture (50 µL) contained 1 µg of genomic DNA. The thermal profile consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 92°C for 1 minute, annealing at 55°C for 30 seconds, extension at 72°C for 3 minutes, and a final elongation step at 72°C for 10 minutes. The PCR products were then verified on a 1.5% (wt/vol) agarose gel in TBE buffer, with ethidium bromide staining, using a λ Hind III digest of lambda DNA as the marker.

Molecular Protocol for Identification of Multi-component Phenol-Hydroxylase Gene

A modified touchdown PCR protocol, as described by Nordlund et al. (1990) and Shingler et al. (1994), was applied to amplify a 209 bp fragment of the phenol-hydroxylase gene. The largest subunit of multi-component phenol hydroxylases (LmPHs) was targeted using the forward primer Phe149GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCGATCGACGAGCTGCGCCA-3') and the reverse primer Phe212 (5'-GTTGGTCAGCACGTACTCGAAGGAGAA-3'). The PCR reactions (50 µL) included 1 µg of genomic DNA and 25 pmol of each primer. The thermal cycling began with a 10-minute activation at 94°C, followed by two cycles at 94°C for 1 minute, 65°C for 1 minute, and 72°C for 2 minutes. Subsequently, the annealing temperature was lowered to 55°C for 20 cycles, with a final extension at 72°C for 10 minutes. The amplified products were confirmed through electrophoresis on a 1.5% (wt/vol) agarose gel in TBE buffer, stained with ethidium bromide, and the fragment sizes were compared against a λ Hind III digest of lambda DNA used as the marker.

Sequence Analysis

The genetic characterization of environmental bacterial strains is crucial for both their accurate taxonomic identification and for understanding their functional capabilities. Among

the six (6) strains, EB demonstrated the greatest ability to break down phenol at an initial concentration of 500 mg/L, 30 °C, 150 rpm and pH of 7. Due to its superior performance in phenol degradation compared to the other isolates, EB was selected for further investigation. In this study, we focused on strain EB and present two key gene sequences; the 16S rRNA gene sequence and the hydroxylase phenol-degrading gene sequence.

The amplified 16S rRNA gene and the corresponding phenol-degrading gene sequences from strain EB were sent to Inqaba biotech™ for sequencing. Once obtained, the sequences were refined using MEGA version 7 and their homology was assessed with the Basic Local Alignment Search Tool (BLAST). The aligned sequences were then automatically integrated into a database, and a phylogenetic tree was constructed using distance matrix analysis through iTOL v7 (Letunic & Bork, 2019). Additionally, database searches and comparisons were carried out using BLAST with the National Centre for Biotechnology Information (NCBI) database (Stephen et al., 1997).

The 16S rRNA gene and the hydroxylase phenol-degrading gene sequences of strain EB not only facilitate its precise identification but also provide a foundation for exploring its biodegradation capabilities. Comparative studies using these sequences can reveal both the phylogenetic context and the metabolic potential of strain EB, thereby supporting its application in environmental clean-up strategies.

Results and Discuss

The isolates designated OKA, IS, IVA, EB, EKA, and ONA were subjected to molecular analysis and the corresponding results are presented below. Figure 1 shows the results of agarose gel electrophoresis for the 16S rRNA gene amplification. Lane L contains the 2 kb DNA ladder, serving as a molecular weight marker for estimating band sizes. The amplified products from all isolates exhibit bands corresponding to the expected 1500 bp fragment of the 16S rRNA gene. The intensity of each band likely corresponds to the abundance of the corresponding DNA fragment in the sample. Brighter bands indicate a higher abundance of that particular 16S rRNA gene sequence.



Figure 1: Agarose Gel Electrophoresis of PCR-Amplified 16S rRNA Gene Gel Products. Lane L: Gene ruler 2 kb DNA ladder markers in bp. All the isolates showed band equivalent to the typical band length of 16S rRNA gene (1500bp).

Figure 2 presents the agarose gel electrophoresis results for the PCR amplification of the monooxygenase gene. Lane L contains the 1 kb DNA ladder, serving as a molecular weight reference. Lanes IS, IVA, and OKA display amplified PCR products, with bands approximately 1,026 bp in size, confirming the successful amplification of the monooxygenase gene in these isolates. The three (3) isolates (IS, OKA and IVA) showed sharp band equivalent to that of mooxygenase gene with band length of 1,026 bp.

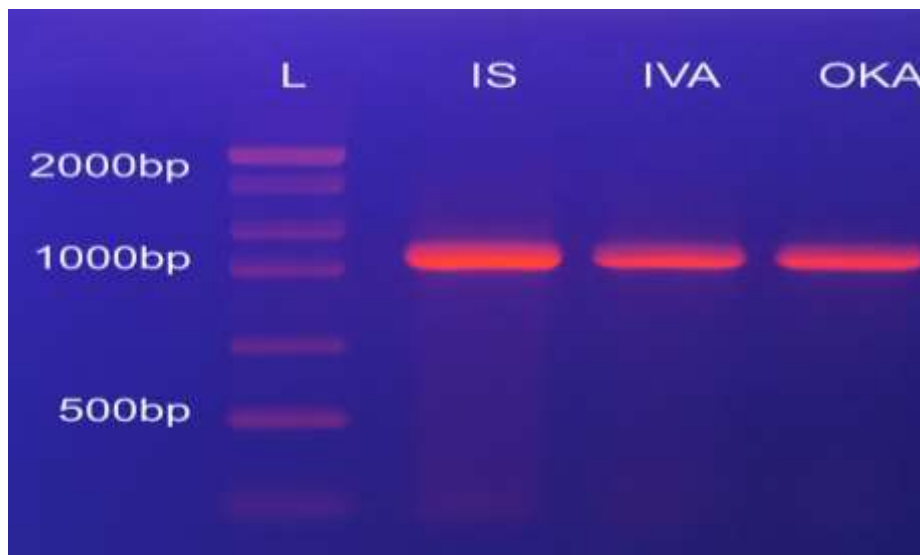
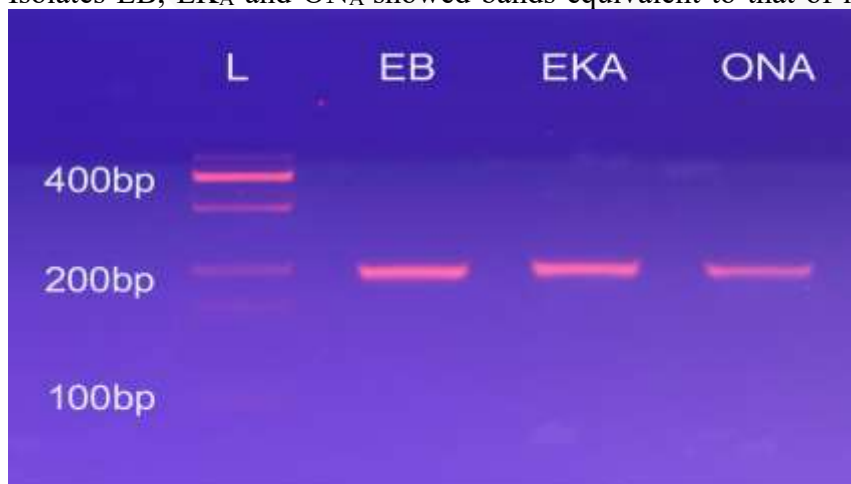


Figure 2: Agarose Gel Electrophoresis of PCR-Amplified Mooxygenase Gene Gel Products. Lane L: Gene ruler 2 kb DNA ladder markers in bp, lane IS, IV_A, and OK_A (Approx. 1,026 bp).

Figure 3 depicts the results of agarose gel electrophoresis for the hydroxylase gene amplification. Lane L contains the 0.4 kb DNA ladder, serving as a molecular weight reference. Lanes EB, EK_A, and ON_A show amplified PCR products with an approximate size of 209 bp, indicating successful amplification of the hydroxylase gene in these isolates. Isolates EB, EK_A and ON_A showed bands equivalent to that of hydroxylase gene with band



length of 209bp.

Figure 3: Agarose Gel Electrophoresis of PCR-Amplified Hydroxylase Gene Gel Products. Lane L: Gene ruler 0.4 kb DNA ladder markers in bp, lane EB, EK_A, and ON_A (Approx. 209 bp).

The Partial 16S rRNA gene sequence of strain EB is:

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ATTCCAGACCCGAAGCACCGCGAGTGGGCCACTACAACAAGTGCTTCAACGGCT
TGGAAGACTGGAACCATAAGCTGCGACCGGGGTGTCTGGTACCTGTCCGTGCCC
AAGTCACTCGAACCAGTGGTTTCGACCGTGTCTGGTACCTGTCCGTGCCCAAGTCT
TTCTTCGAGGGGCATGCACAACCTCGAACTTTCTTCGAGGGTCCGTACCTGTCCGT
GCCCAAGTCTTTCTTGGAAGATGCCGGAACCCGGCCTTTCGAGTTCCTGATGGCC
ATCAGCTTCTCCTTCGAGTACGGGGCGGGTACCG
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The partial hydroxylase phenol-degrading gene sequence of strain EB is:

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AGGCGGGTGGACAATGGGCGCAAGCCTGATCCAGCCATTCCGCGTGAGTGAAGA
AGGCCTTCGGGTGTAAAGCTCTTTCGCAAGGGAAGAAAACCTTAGGCTCTAACAT
ATTGAACGCTAGCGGGATGCTTTACACATGCAAGTCGAACGGCAGCGCGAGAGA
GCTTGCTCTCTTGGCGGCGAGTGGCGGACGGGTGAGTAATATATCGGAACGTGC
CCAGTAGCGGGGGATAACTACTCGAAAGAGTGGCTAATACCGCATAACGCCCTAC
GGGGGAAAGGGGGGGATCGCAAGACCTCTCACTATTGGAGCGGCCGATATCGGA
TTAGCTAGTTGGTGGGGTAAAGGCTCACCAAGGCAACGATCCGTAGCTGGTTTG
AGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCG
TGTATGATGAAGGCCTTCGGGTGTAAAGTACTTTTGGCAGAGAAGAAAAGGTA
CTCCTAATACGAGNTACTGCTGACGGTATCTGCAGAATAAGCACCGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGTGTGTAGGCGAGCCTGAGGTTGACGGTACCTTGATAAGAAGCACC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAATCGG
AATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGTCAGATGTGAAATCC
CCGAGCTCAACTTGGGAAGTGCCTTTGAACTACAAGACTAGAGTATGTCAGAG
GGGGGTAGAATTCCATGTGTAGCAGTGAAATGCGCAGAGATGTGGAGGAATACC
AATGGCGAAGGCAGCCCCCTGGGATAATATTGACGCTCATGCACGAAAGCGTGG
AAACTCTAACA.
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The nucleotide sequence above was compared to those on the NCBI Gene Bank database. Strain EB exhibited 99.8% 16S rRNA sequence identity with *Alcaligenes faecalis* and *Alcaligenes phenolicus*. More so, strain EB clusters most closely (97.22% sequence identity) with the other phenol degrading *Alcaligenes* strains indicating a strong functional and evolutionary relationship in their phenol-degrading gene sequences and the phylogenetic trees were constructed using iTOL v7. The tree in Figure 4 shows the placement of *Alcaligenes* sp. EB among several *Alcaligenes* species, with *Bordetella bronchiseptica* serving as a more distant reference (or outgroups). The branch lengths (indicated by the scale bar) reflect inferred genetic distances. Notably, *Alcaligenes* sp EB clusters among known *Alcaligenes faecalis* and *Alcaligenes phenolicus* strains, demonstrating its close evolutionary relationship to these taxa based on their 16S rRNA gene sequences.

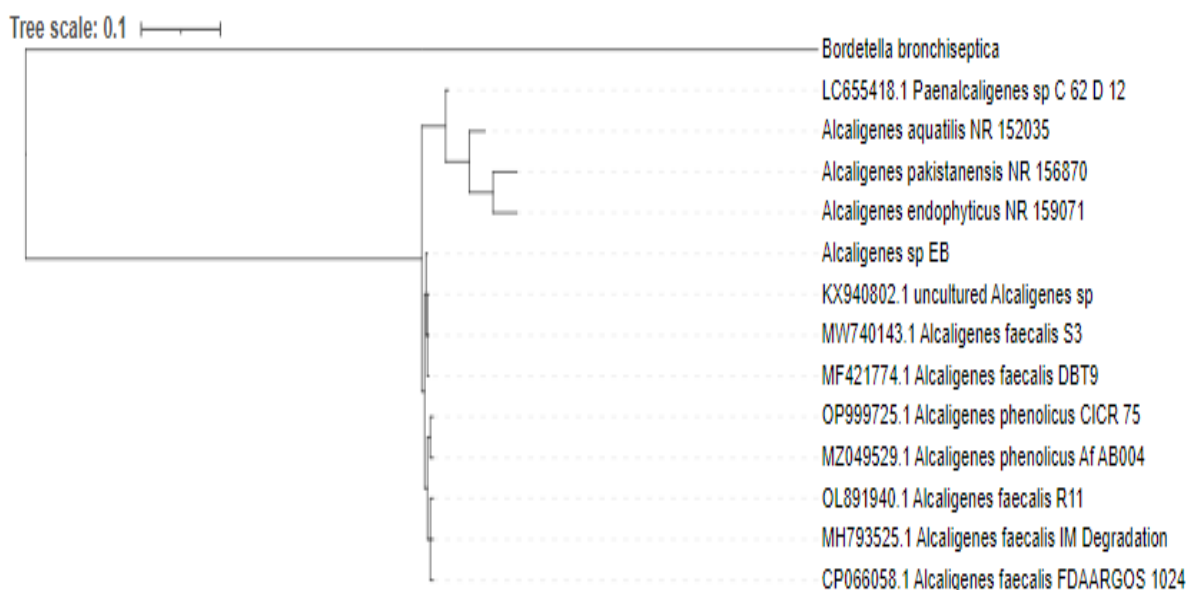


Figure 4: 16S rRNA-Based Phylogenetic Tree of Strain EB within the *Alcaligenes* Genus
The tree in Figure 5 illustrates the relationships among bacterial strains based on their multi-component hydroxylase phenol-degrading gene sequences. Two uncultured *Marinobacterium* isolates form one branch, while *Pseudomonas aeruginosa*, *Sphingobium* sp. X-b4, and *Arthrobacter* sp. W1 cluster separately. *Alcaligenes* sp. EB groups with other *Alcaligenes* strains, underscoring their close functional and evolutionary similarity in phenol degradation pathways.

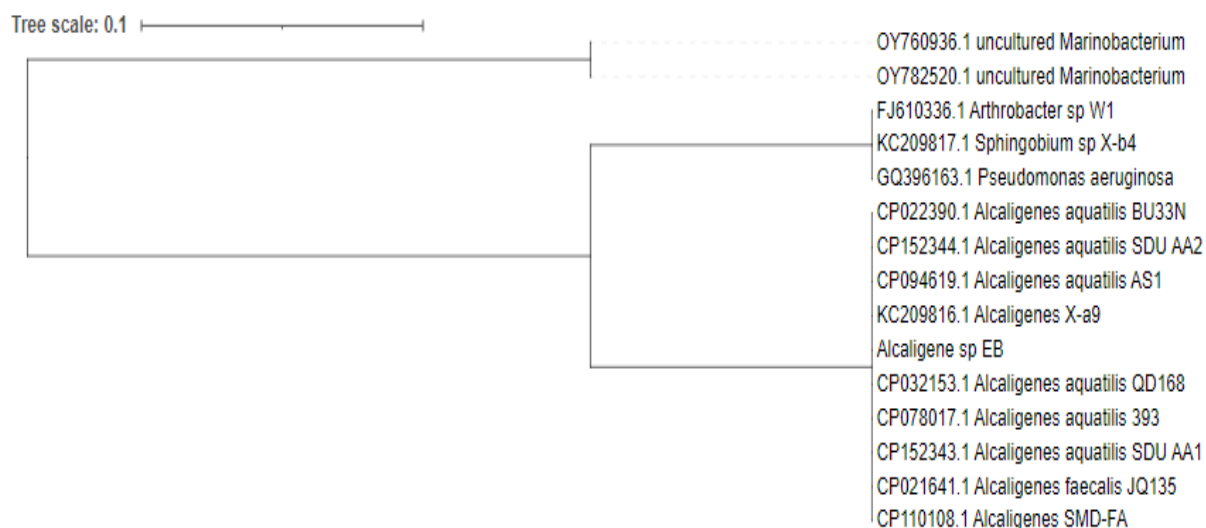


Figure 5: Phylogenetic Tree Illustrating the Hydroxylase Phenol-Degrading Gene Sequences of Strain EB among Diverse Bacterial Strains.

The 16S rRNA gene analysis clearly places strain EB within the *Alcaligenes* genus (Figure 4), while phylogenetic examination of its phenol-degrading genes shows that they group most closely with those from other *Alcaligenes* strains (Figure 5).

Consequently, strain EB has been provisionally designated as *Alcaligenes* sp. EB with an accession number of PV536963.

Discussion

In the current study, the six (6) phenol-degrading bacterial isolates (IS, OKA, IVA, EB, EKA, and ONA) that originated from the hydrocarbon-polluted soils of Gombe metropolis were examined for their molecular and functional characteristics. The results of the study are very important not only for understanding the genetic identity, the metabolic pathways and the phylogenetic relationships of the isolates but also for the phenol-contaminated environmental bioremediation in a wider context. The high levels of phenol in the soil over Gombe metropolis that were noted before by Lazarus et al. (2025) draw attention to the environmental stress due to industrial activities, automobile repairs, fuel handling and improper waste disposal. The presence of phenol is a serious problem due to its toxicity, persistence and potential for bioaccumulation up the food chain (Agency for Toxic Substances and Disease Registry, 2022; Zhang et al., 2011). The recovery of phenol-degrading bacteria from these soils further substantiates the idea that indigenous microbial communities acquire specialized metabolic capabilities through long-term exposure to pollutants, which is the case in the study of Basak et al. (2014).

The successful amplification of the ~1500 bp 16S rRNA gene in all isolates is a clear indication that the genomic DNA extracted was pure and suitable for further molecular applications. The universal amplification of this gene is in agreement with the findings of previous studies which reported the gene's high conservation among bacterial taxa and its effectiveness for phylogenetic identification (Weisburg et al., 1991). Moreover, the presence of PCR bands that were sharp and well-defined imply that the isolates were indeed active and stable both metabolically and genetically during the whole processes of enrichment and cultivation. Differential amplification of the genes encoding monooxygenase and hydroxylase unveiled functional diversity of the isolates. The isolates IS, OKA, and IVA yielded the expected ~1026 bp fragments of the monooxygenase gene which is in line with their positive indole hydroxylation phenotype. Monooxygenases perform the first hydroxylation of phenol to catechol which is a critical rate-limiting step in phenol degradation (Shingler et al., 1992). The fact that this gene exists in these isolates suggests that they may be able to carry out phenol catabolism via monooxygenase-mediated ortho- or para-hydroxylation pathways, something that has been previously described in bacteria like *Pseudomonas*, *Arthrobacter*, and *Enterobacter* (Kaneekar et al., 1999; Shebl et al., 2024).

Conversely, the isolates EB, EKA, and ONA had the ~209 bp multi-component phenol hydroxylase (mPH) gene fragment, implying their dependence on a multi-subunit hydroxylase system like those of organisms that thrive in phenol and cresol catabolism. Multi-component phenol hydroxylases exist in soil bacteria and are characterized by a broad substrate range, besides being highly efficient in the degradation of phenolic xenobiotics (Watanabe et al., 2002). The mPH-encoding microorganisms reported to be in contaminated habitats (Long et al., 2016; Hoyos-Hernandez et al., 2014) are the ones that the isolates' ability to utilize phenol as a solitary carbon source agrees with. Amongst all the bacterial isolates, strain EB had the highest ability to degrade phenol and hence it was chosen for genetic sequencing. Its 16S rRNA sequence was found to be 99.8% identical to that of *Alcaligenes faecalis* and *Alcaligenes phenolicus*, therefore confirming its being part of the *Alcaligenes* genus. This is in line with the widely accepted knowledge on the biodegradation potential of *Alcaligenes* species, the majority of which can utilize not only phenolic compounds but also aromatic hydrocarbons (Basak et al., 2014). Phylogenetic studies using hydroxylase gene sequences also showed that strain EB is very near to other phenol degrading *Alcaligenes* strains. This very close relationship in evolutionary terms highlights the fact that catabolic pathways for aromatic degradation are conserved among such genera. EB being aligned with the taxa that have multi-component hydroxylase systems is in

agreement with its phenotypic and molecular characteristics, as reported by Lazarus et al., (2025) and, therefore, its functional role in phenol mineralization is being supported.

The detection of phenol-catabolizing genes in all six isolates reflects adaptive microbial responses to phenolic contamination in Gombe metropolis. The enrichment of bacteria capable of degrading phenol aligns with findings from similar hydrocarbon-impacted environments, where selective pressure favours organisms equipped with catabolic pathways for aromatic compounds (Zhang et al., 2011; Basak et al., 2014). Given the documented hazards of phenol including its toxicity, environmental persistence, and impact on soil and groundwater (Agency for Toxic Substances and Disease Registry, 2022; International Agency for Research on Cancer, 2020), the discovery of multiple indigenous phenol degraders presents promising opportunities for bioremediation. The diverse enzymatic pathways identified (monooxygenase and multi-component hydroxylase systems) suggest robust biodegradation potential across varying environmental conditions. Furthermore, the dominance of efficient degraders such as *Alcaligenes* sp. EB supports its potential application in bio-augmentation strategies aimed at restoring phenol-contaminated soils in the region.

Conclusion

This study demonstrates the presence of taxonomically and functionally diverse phenol-degrading bacteria in hydrocarbon-contaminated soils of Gombe metropolis. The molecular evidence confirms that isolates employ distinct catabolic strategies for phenol degradation, with strain EB emerging as a highly efficient degrader phylogenetically affiliated with known *Alcaligenes* species. The findings highlight the substantial natural bioremediation potential of indigenous microbial communities in mitigating phenolic pollution and provide a foundation for future applied bioremediation research.

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