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Isolation and Molecular Identification of Bacillus Subtilis in Petroleum-Contaminated Soil

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Abstract

Petroleum pollution poses a significant threat to soil health and ecosystem stability, particularly in oil-producing regions. This study aimed to isolate and identify hydrocarbon-degrading bacteria from contaminated soil samples. Four soil samples were collected, preserved, and analyzed for microbial growth. The isolate with the highest colony-forming unit count was subjected to Gram staining, biochemical tests, and molecular identification using 16S rRNA gene sequencing. The isolate with the highest colony-forming unit count was subjected to Gram staining, biochemical tests, and molecular identification using 16S rRNA gene sequencing. The bacterium was confirmed as Bacillus subtilis, showing positive catalase and motility reactions, and negative oxidase activity. BLAST analysis revealed 100% sequence identity with B. subtilis strain 17A. These findings validate the potential of B. subtilis as a native bioremediation agent for petroleum-contaminated soils, offering a sustainable solution for environmental restoration.

Keywords: B. subtilis; petroleum-contaminated[1] soil; bioremediation.

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1. Introduction

Petroleum contamination of soil is a widespread environmental challenge with significant ecological, economic, and public health implications. The global demand for petroleum and its derivatives has led to extensive exploration, refining, transportation, and usage, all of which contribute to accidental spills, leakages, and chronic pollution of terrestrial ecosystems[1] .Globally, petroleum-polluted soils are most prevalent in oil-producing regions such as the Middle East, North America, Russia, and sub-Saharan Africa[1,4].Some challenges are reported in Alaska (Exxon Valdez spill), the Gulf of Mexico (Deepwater Horizon disaster), and other industrial zones where petroleum activities are concentrated[1, 5] .The persistence of hydrocarbons, particularly polycyclic aromatic hydrocarbons (PAHs), makes remediation difficult, as these compounds are resistant to natural degradation and can remain in soils for decades.

In Nigeria, crude oil spills, pipeline leakages, illegal refining, and poor waste management practices have led to widespread contamination of soil and groundwater[4].These actions have continually disrupted ecosystems and threatened livelihoods. The hydrocarbons in petroleum alter soil chemistry, reducing fertility, impairing microbial activity, and making the land unsuitable for agriculture[2-4, 6].Studies show that oil-polluted soils often exhibit high concentrations of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which persist for long periods and bioaccumulate in plants and animals[2-4, 6].This contamination not only undermines food security but also exposes local populations to health risks, including respiratory problems, cancers, and reproductive disorders[3].

Petroleum contamination influences soil contamination, soil pollution, and land degradation, which are basically caused by the presence of xenobiotic (human-made) chemicals and alterations in the natural soil environment. This soil deterioration is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, polynuclear aromatic hydrocarbons, solvents, pesticides, lead, and other heavy metals [7].

Petroleum exploitation and use have substantial negative environmental and ecological consequences[3].Also, conventional remediation methods, such as excavation, incineration, and chemical treatment, are costly and often environmentally disruptive. As a result, global attention has shifted toward bioremediation, which harnesses the metabolic capabilities of microorganisms to degrade hydrocarbons into less harmful compounds. Indigenous soil bacteria, including species of *Bacillus*, *Pseudomonas*, and *Acinetobacter*, have been shown to adapt to petroleum-polluted environments and utilize hydrocarbons as energy sources. In communities dependent on farming and fishing, petroleum pollution has contributed to poverty, displacement, and social unrest[2, 3].Furthermore, remediation efforts have been slow and inadequate, with many sites remaining untreated decades after spills. While bioremediation and phytoremediation techniques have shown promise, their implementation has been limited by poor regulatory enforcement and a lack of funding. Furthermore, *Bacillus subtilis* interactions with compounds and other content of petroleum hydrocarbons have been reported to bioremediate soil and balance the ecological and biological setting of the affected area [6,10].

Bacillus subtilis plays an important role as a bioremediation agent due to its metabolic versatility and resilience

in harsh environments. Researchers have established that this petroleum contamination influences the activities of the bacteria *Bacillus subtilis*, which is also known as the hay *Bacillus* or grass *Bacillus.* [6, 8,10] *B. subtilis* is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants, humans, and marine sponges [11,13]. Being a species of the genus *Bacillus*, *B. subtilis* is rod-shaped and forms a tough, protective endospore, which enables it to exist even in extreme environmental conditions, e.g., on petroleum-contaminated soil.

Moreso, they are one of the bacterial kings for their secretion of enzyme production, notable for industrial application by biotechnology companies [11, 13]. This bacterium produces a wide range of extracellular enzymes and biosurfactants that enhance the breakdown of hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs) and other toxic petroleum derivatives. By secreting biosurfactants such as surfactin, *B. subtilis* increases the solubility and bioavailability of hydrophobic petroleum compounds, allowing microbial communities to degrade them more efficiently. Its ability to form endospores also makes it highly resistant to environmental stress, enabling survival and activity in contaminated soils with fluctuating pH, temperature, and nutrient levels[11, 13].

In Nigeria, where fertility is compromised and microbial diversity is reduced, *B. subtilis* contributes to restoring ecological balance by stimulating microbial consortia, improving soil structure, and supporting plant growth through its plant growth–promoting traits. Moreover, its non-pathogenic nature and widespread occurrence make it a safe candidate for bioaugmentation strategies aimed at cleaning up oil-impacted sites. Thus, this research investigates the role of *B. subtilis* by isolating from *B. subtilis* from petroleum-contaminated soil and validating its molecular identification as well. This facilitates the understanding of *B. subtilis'* ecological and practical impacts, which are detoxification of polluted soils, enhancement of agricultural productivity, and support for sustainable remediation efforts in regions heavily affected by petroleum contamination. Hence, this research examines and identifies *B. subtilis* in a soil contaminated by a petroleum product. The objectives are:

- To isolate B. subtilis using the pour plate inoculation technique.
- To identify and confirm B. subtilis using biochemical tests.
- To identify and confirm B. subtilis using the Polymerase chain reaction (PCR) technique.

2. Methods

The soil samples were obtained from Ogoni land in the Niger Delta region. The practical was performed at the Cellulose Laboratory, Abuja, Nigeria, and the PCR test was conducted at the Biology Lab, Department of Natural Science, New Mexico Highland University. The practical study involved the collection, identification, and isolation of *Bacillus subtilis*, and the molecular identification.

2.1 Isolation of Bacillus subtilis

Isolation was performed using the pour plate method[14].One gram of contaminated soil was serially diluted to 10^{-6} in sterile distilled water. From the 10^{-2} dilution, 1 mL was plated with molten nutrient agar and incubated at 37 °C for 24 h. Plates with 30–300 colonies (cfu/g) were selected, and subcultures were prepared on fresh nutrient agar for identification.

2.2 Identification of Bacillus subtilis

Identification involved microscopic, biochemical, and molecular techniques.

1. Gram Staining: Smears of the bacterial isolates were prepared on grease-free slides, heat-fixed, and sequentially stained with crystal violet, iodine, alcohol decolorizer, and safranin. After air-drying, slides were examined under oil immersion. The isolates appeared Gram-positive, consistent with *B. subtilis*.[8]
2. Biochemical Tests: The biochemical characterization of the isolate was carried out using standard tests, including catalase, motility, and oxidase assays[8, 15] .In the catalase test, a purified bacterial culture was exposed to hydrogen peroxide, and the immediate release of gas bubbles within 10 seconds confirmed a positive reaction, consistent with *Bacillus subtilis*[8, 15].Motility was assessed by preparing wet mounts from nutrient agar subcultures and peptone broth; microscopic examination revealed active movement of cells, indicating motility[8, 15, 16].For the oxidase test, colonies were smeared onto filter paper saturated with freshly prepared oxidase reagent, and the rapid development of a purple coloration within 10 seconds demonstrated oxidase activity[8, 15, 16].
3. Molecular Identification (PCR): DNA was extracted from the sample using the Zymo Quick DNA Miniprep Kit. Approximately 70 mg of bacterial cells were lysed in ZR Bashing Bead™ tubes, centrifuged, and purified through Zymo-Spin™ columns. DNA integrity was confirmed by agarose gel electrophoresis, and purity was assessed with NanoDrop spectrophotometry. PCR amplification targeted the 16S rRNA gene using universal primers 27F and 1492R. Reactions were run in a thermal cycler with 40 cycles of denaturation (94 °C, 1 min), annealing (40 °C, 1 min), and extension (72 °C, 1 min), followed by a final extension at 72 °C for 10 min. Amplified products were separated on 1% agarose gel, visualized under UV, and compared against a 1 kb DNA ladder.

3. Results

3.1 Bacterial Colony Count of the Isolates

A comparative analysis of bacterial colony counts was conducted across four soil samples, A, B, C, and D, each exposed to varying levels of petroleum contamination. The colony-forming units per gram (cfu/gm) were quantified to assess microbial proliferation under petroleum contamination. The results are summarized in Table 1.

Table 1: Bacterial Colony Count of the Soil Samples

Sample	Count (cfu/gm)
A	216
B	236
C	352
D	172

Sample C exhibited the greatest bacterial count with 352 cfu/gm. Sample B had 236 cfu/gm, while Sample A had 216 cfu/gm. The lowest count was observed in Sample D, with 172 cfu/gm,

3.2 Gram Staining Test Result

From the contaminated soil samples, the selected isolate with the highest colony-forming count was characterized macroscopically by its rough texture, fuzzy appearance, creamy-white coloration, circular shape, flat elevation, and entire margin. This single colony was sub-cultured on freshly prepared nutrient agar and incubated at 37 °C for 24 hours to obtain a pure culture. Gram staining of the isolate revealed rod-shaped cells that retained the purple coloration of the primary crystal violet stain, indicating a Gram-positive reaction. This microscopic observation confirmed the isolate as a Gram-positive bacillus, consistent with the characteristics of *Bacillus subtilis*. The Gram-stained view and morphology of the isolate are shown below.

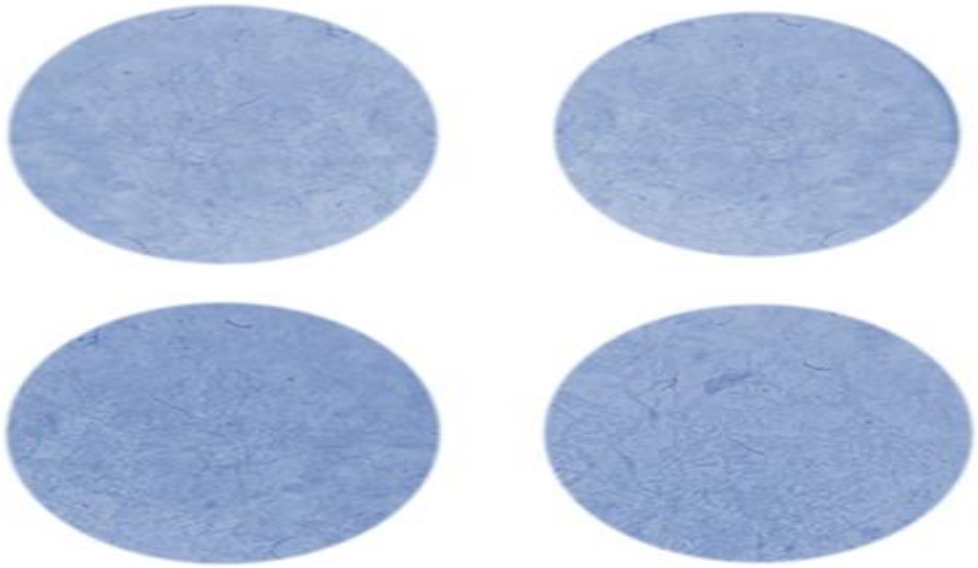

Figure 1: Microscopic view of the isolates

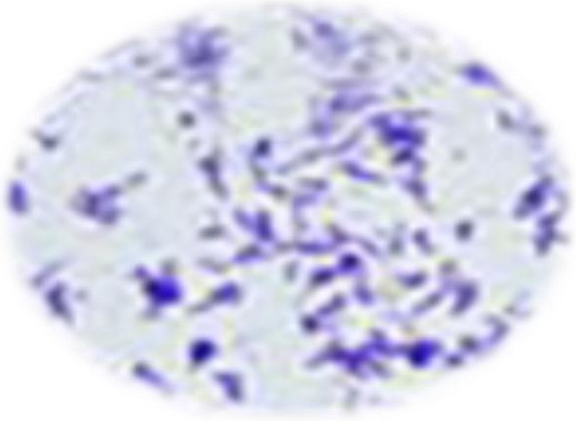

Figure 2: Microscopic view of the isolate

Table 2: Gram staining test reaction

Isolate	Size	Gram stain	Color	Form
B. subtilis	Rods	+	Purple	Circular flat

3.3 Biochemical Test Results

Biochemical characterization of the selected bacterial isolate was conducted to support its identification as *Bacillus subtilis*. Three standard tests, catalase, motility, and oxidase, were performed, and the outcomes are summarized in Table 3.

Table 3: Biochemical test results

Test	Reaction
Catalase	Positive (+)
Motility	Positive (+)
Oxidase	Negative (–)

The isolate demonstrated a positive catalase reaction, evidenced by the rapid formation of gas bubbles upon exposure to hydrogen peroxide. This indicates the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen, a characteristic trait of aerobic organisms like *B. subtilis*. The motility test also returned a positive result, with active movement observed under the microscope, confirming the presence of flagella and the organism's ability to move independently. Conversely, the oxidase test was negative, as no color change occurred within the expected time frame. This result indicates the absence of cytochrome c oxidase, which is consistent with the biochemical profile of *Bacillus subtilis*.

3.4 Polymerase Chain Reaction Identification Results

3.4.1Genomic DNA Extraction and Quality Assessment

Genomic DNA was successfully extracted from the selected *Bacillus subtilis* isolate using the Zymo Quick DNA Fungal/Bacterial Miniprep Kit. The integrity and quality of the extracted DNA were assessed via agarose gel electrophoresis. As shown in *Figure 3*, Lane D displayed a distinct, high-molecular-weight band near the top of the gel, indicating intact genomic DNA with minimal fragmentation. Lane A, which contained the DNA ladder, served as a reference for determining the size of the DNA fragments. The clarity and intensity of the band confirmed that the DNA was suitable for PCR and sequencing.

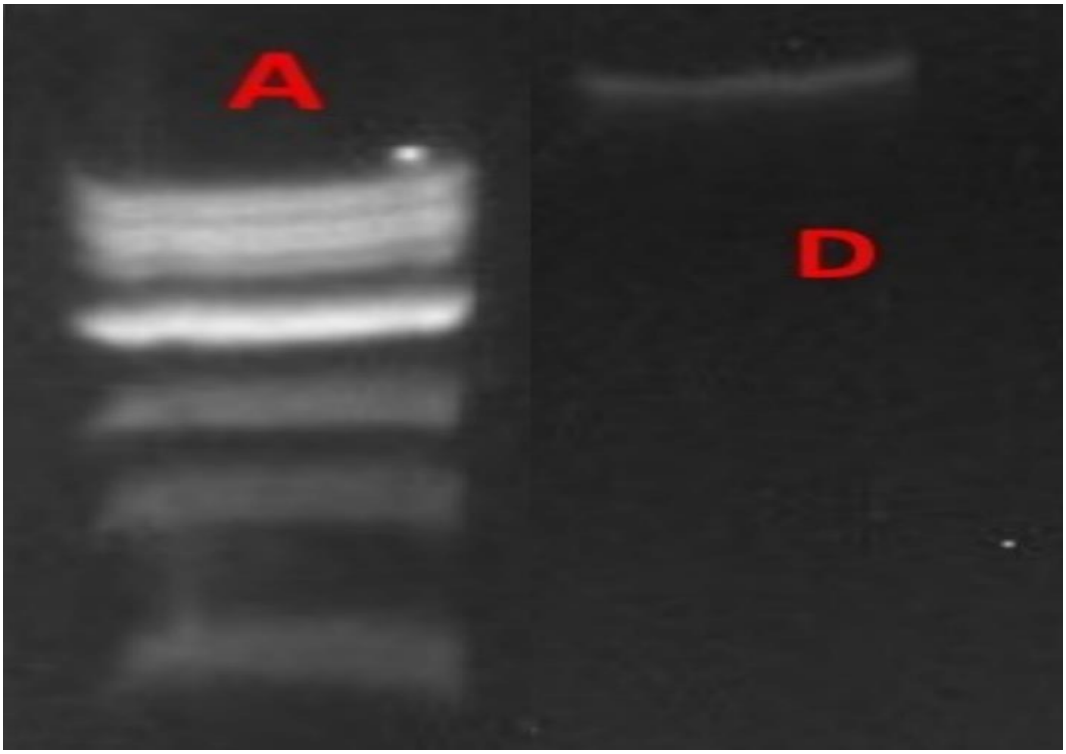

Figure 3: Genomic DNA extraction of *B. subtilis* isolate

3.4.1 PCR Result of Bacillus subtilis

Polymerase Chain Reaction (PCR) analysis was conducted to confirm the molecular identity of the bacterial isolate obtained from the selected sample. Amplification of the 16S ribosomal RNA gene was performed using universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′). The presence of distinct DNA bands corresponding to the expected size of the 16S rRNA gene confirmed successful amplification. The band pattern observed in Figure 4 is consistent with *Bacillus subtilis*, validating its molecular identity and supporting previous biochemical and microscopic findings.

GGCGTGCTATACATGCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGC
GGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATAC
CGGATGCTTGTTTGAACCGCAAGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCG
CGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCTACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAGYMGTAGGGAATSTWCCGCAATG
GACGAAAGTSYGACGGAGCAACGCCGCGTGAGTGRWGWARGTTTTCGGATCGTAAAGCTCWGTTGTTASG
GAARAACARGTACCGTWCGAATAGKGCSGTACCTTGACSGTACCTWACCAGAWAGCCACGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTMCKGAATTATTGGGCGTAAWGGGCTCGCARG
CGGTTCSTTAAAGTCTGATGTGAAAGCCCCSGGCTCAACCGGGGARGGTCATTSGAAACTGGCGRAACTT
GAGTGCAGAAGAGGAGTAGTGGAAATTYCACGTGTAGCGGTGAAATGCGTAGAGATGTGTGAGGAACACC
AGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA
GATACCCTGGTAGTMCACGCCGTAAACGATGAGTGCTAAGTGATTAGGGGGGTTTCCGCCCCTTAGTGCT
GCAGCTRACGCATTAAGCACTCCGGCCTGGGGAGTACRGTCGCAAGACTGATAACTCAAAGGAATTGACG
GGGGCCCGCACAAGGCGGTGGAGCATGATGGTTTAATTCGAATGCAACGCGAAGAATCMTTACCAAGKTC
TTGACWTCCTCTGACAWTCSTAGAAGATAGGACGTCCTCCATTCSGGGGCAKASTGACATGKTCGRTGCA
TTGSTTKTTCGTCAGCATTCGTTGTTCGTGAGAATTCGTTGGRATTTAMGTTCCCGCAATCGAGCGCARC
CMTTGAATTCTTTASTTGGCYAGCATTCAGTTGGGCACTCTYAAGKTGACCTGMCGGTGACAAACCGGAG
KAMGGTSGGGATKACGTYMAATCTATCATKCCCCKTATGACCTTGGCTACACACCTGCTACAATGGACAG
AACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCCCAAATCTGTTTTCAGTTCGGATCGCAGTTTG
CAACTTGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTATGG
AGCCAGCCGCCGAAGGTGGGACACGATGATTGGGGTGAAGTCGTAACAAGTAGCCATAGTTGC

Figure 4: PCR Band Pattern of the extracted DNA of *B. subtilis* isolate

3.4.2 BLAST Analysis of 16S rRNA Gene Sequence

Following successful amplification of the 16S ribosomal RNA gene from the bacterial isolate, the resulting sequence was subjected to BLAST (Basic Local Alignment Search Tool) analysis to confirm species-level identity. The sequence was aligned against the NCBI nucleotide database, and the top hit returned a 100% match with *Bacillus subtilis* strain 17A, as shown in Figure 5.

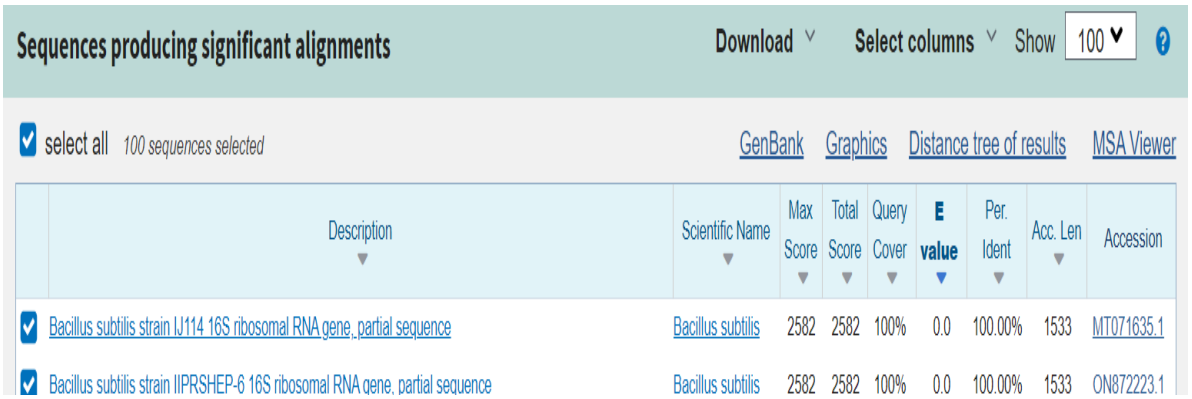

Figure 5: Gene Sequencing of the extracted DNA of *B. subtilis* isolate

These results indicate a perfect alignment between the query sequence and the reference strain, confirming the isolate as *Bacillus subtilis*.

4. Discussion

The successful isolation and identification of *Bacillus subtilis* from petroleum-contaminated soil in this study validate its role in environmental bioremediation. The isolate was confirmed through Gram staining, biochemical tests, and molecular analysis. The Gram-positive, rod-shaped morphology, along with positive catalase and motility reactions and negative oxidase activity, aligns with the known biochemical profile of *B. subtilis*[16, 17]. Molecular confirmation via 16S rRNA gene sequencing and BLAST analysis further validated the identity of the isolate, showing 100% sequence similarity with *Bacillus subtilis* strain 17A.

These findings are consistent with those of Al-Dhabaan, who reported that 16S rDNA fingerprinting reliably confirmed *B. subtilis* identity in similar environmental samples[8].The ability of *B. subtilis* to interact and habituate in petroleum-polluted soil establishes its adaptive resilience and metabolic versatility. Additionally, Tabari demonstrated that biosurfactants produced by *B. subtilis* are stable across a wide range of environmental conditions, including temperature, salinity, and pH (5–12), and are capable of degrading aliphatic hydrocarbons[18].This supports that *B. subtilis* isolated possesses biosurfactant-producing capabilities, contributing to its survival and activity in hydrocarbon-rich environments. Further evidence of *B. subtilis*'s bioremediation potential is provided by Bekele and his colleagues, who reported that indigenous bacterial isolates, including *B. subtilis*, achieved up to 91% kerosene degradation within 15 days[19].

Hosseini-Boldaji and his colleagues also emphasized the role of *B. subtilis* in decomposing complex oil alkanes into simpler compounds, with the presence of amine and carboxyl functional groups detected in the culture medium[16].Their findings support the use of biological methods as viable alternatives to conventional physical and chemical remediation techniques. The isolation of native soil bacteria, such as *B. subtilis*, that have adapted to petroleum exposure is particularly valuable for developing sustainable, low-cost bioremediation approaches suitable for local environmental conditions.

Above all, the results of this study and supporting literature confirm that *Bacillus subtilis* is an agent for bioremediation of petroleum-contaminated soils. Its ability to survive, proliferate, and metabolize hydrocarbons under stress conditions makes it a promising agent for environmental restoration in regions like the Niger Delta, where oil pollution remains a persistent challenge.

5. Conclusion

This study has investigated and successfully isolated and identified *Bacillus subtilis* from petroleum-contaminated soil. The isolate was confirmed through Gram staining, biochemical tests (positive catalase and motility, negative oxidase), and molecular analysis via 16S rRNA gene sequencing. BLAST results showed 100% identity with *B. subtilis* strain 17A, validating the species-level identification. The integration of native microbial isolates like *B. subtilis* into bioremediation strategies offers a sustainable alternative to conventional methods, particularly in oil-impacted regions such as Nigeria's Niger Delta.

References

[1] D. Pal and S. Sen, "In-Depth Coverage of Petroleum Waste Sources, Characteristics, Environmental Impact, and Sustainable Remediation Process," in *Impact of Petroleum Waste on Environmental Pollution and its Sustainable Management Through Circular Economy*, I. D. Behera and A. P. Das Eds. Cham: Springer Nature Switzerland, 2023, pp. 1–38.

[2] M. A. Adeniran, M. A. Oladunjoye, and K. O. Doro, "Soil and groundwater contamination by crude oil spillage: A review and implications for remediation projects in Nigeria," (in English), *Frontiers in Environmental Science*, Review vol. Volume 11 - 2023, 2023–May–09 2023, doi: 10.3389/fenvs.2023.1137496.

[3] B. O. Akagbue, M. a. Aminu, Baba, S. Babatunde, T. O. Popoola, and J. A. Nenger, "Negative Health and Environmental Effects of Oil Exploitation in Southern Ijaw, Bayelsa State, Nigeria," *European Journal of Environment and Earth Sciences*, vol. 5, no. 3, pp. 34–41, %06/%21 2024, doi: 10.24018/ejgeo.2024.5.3.464.

[4] D. Onuoha, J. Aso, and U. Okolie, "A critical review of the effects of oil spillage on land in Nigeria," *Int J Agricult Earth Sci*, vol. 9, no. 7, pp. 2695–1894, 2023.

[5] I. I. George, M. G. M. Nawawi, Z. J. Mohd, and B. S. Farah, "Environmental effects from petroleum product transportation spillage in Nigeria: a critical review," *Environmental Science and Pollution Research*, vol. 31, no. 2, pp. 1719–1747, 2024/01/01 2024, doi: 10.1007/s11356-023-31117-z.

[6] B. M. Popoola, B. V. Oluwatusin, and O. J. Olayiwola, "Qualitative Assessment of Biosurfactant Producing Microorganisms in the Cleanup of Crude Oil in Soil," *Ajayi Crowther Journal of Pure and Applied Sciences*, vol. 2, no. 1, 2023.

[7] R. George, J. Varsha, S. Aiswarya, and P. Jacob, "Treatment methods for contaminated soils-translating science into practice.," *Intrnational Journal of Education and Applied Research* pp. 4(1): 17–19, 2016.

[8] F. A. Al-Dhabaan, "Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saud Arabia," *Saudi Journal of Biological Sciences*, vol. 26, no. 6, pp. 1247–1252, 2019.

[9] A. N. Al-Najim and E. G. Alsammak, "Effect of Mersacidin produced from Bacillus sp.-AE isolated from soils in Northern Iraq," *Journal of Survey in Fisheries Sciences*, vol. 10, no. 2S, pp. 1914–1928, 2023.

[10] G. K. Bekele *et al.*, "Kerosene Biodegradation by Highly Efficient Indigenous Bacteria Isolated From Hydrocarbon-Contaminated Sites," *Microbiology Insights*, vol. 16, p. 11786361221150759, 2023.

[11] S. I. Paul and M. M. Rahman, "Draft Genome Sequence of Bacillus subtilis YBS29, a Potential Fish Probiotic That Prevents Motile Aeromonas Septicemia in Labeo rohita," (in eng), *Microbiol Resour Announc,* vol. 11, no. 10, p. e0091522, Oct 20 2022, doi: 10.1128/mra.00915-22.

[12] S. I. Paul, M. M. Rahman, M. A. Salam, M. A. R. Khan, and M. T. Islam, "Identification of marine sponge-associated bacteria of the Saint Martin's island of the Bay of Bengal emphasizing on the prevention of motile Aeromonas septicemia in Labeo rohita," *Aquaculture,* vol. 545, p. 737156, 2021/12/15/ 2021, doi: https://doi.org/10.1016/j.aquaculture.2021.737156.

[13] M. M. Rahman, S. I. Paul, T. Akter, A. C. Y. Tay, M. J. Foysal, and M. T. Islam, "Whole-Genome Sequence of Bacillus subtilis WS1A, a Promising Fish Probiotic Strain Isolated from Marine Sponge of the Bay of Bengal," (in eng), *Microbiol Resour Announc,* vol. 9, no. 39, Sep 24 2020, doi: 10.1128/mra.00641-20.

[14] U. Yusuf, I. Ekeleme, E. Owuna, S. Ochai, and S. Obiekezie, "Effects of spent hydrocarbon on bacteria population," *International Journal of Scholarly Research in Science and Technology.* , vol. 2. , pp. 001–009., 2023.

[15] A. Tankeshwar. "Tests for Bacterial Motility: Procedure, Results." Microbe online. https://microbeonline.com/?s=test+for+bacterial+motility (accessed 2023).

[16] S. A. Hosseini-Boldaji, H. Soltani, and M. Teimouri, "Isolation and purification of petroleum-decomposing bacteria from contaminated soils and identification the resulted compounds of degradation of such compounds," *Applied Biology,* 2023.

[17] S. Valizadeh, N. Enayatizamir, H. N. Ghomsheh, H. Motamedi, and B. K. Moghadam, "Characterization of the biosurfactant production and enzymatic potential of bacteria isolated from an oil-contaminated saline soil," *International Microbiology,* pp. 1–14, 2023.

[18] K. Tabari and M. Tabari, "Characterization of a biodegrading bacterium, Bacillus subtilis, isolated from oil-contaminated soil," *International Journal of Environmental Science and Technology,* vol. 14, no. 12, pp. 2583–2590, 2017/12/01 2017, doi: 10.1007/s13762-017-1313-3.

[19] G. Bekele *et al.*, "Kerosene Biodegradation by Highly Efficient Indigenous Bacteria Isolated From Hydrocarbon-Contaminated Sites " *Sage,* pp. 1–9, 2023.