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Application of *Priestia megaterium* in the Bioremediation of Petroleum Hydrocarbon Contaminated Environments

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Abstract

Petroleum pollutants pose a serious and persistent threat to environmental ecosystems, particularly affecting soil and water quality. Effective and sustainable remediation strategies are crucial to mitigate their impact and safeguard environmental health. In this context, bioremediation – using microorganisms to degrade harmful contaminants – emerges as a promising and eco-friendly approach. The present study explores the biodegradation capabilities of the bacterium Priestia megaterium, a species previously recognized for its ability to break down hydrocarbons. The primary objective of this research is to isolate and characterize Priestia megaterium from soil samples obtained from areas near a petrol pump, a site typically subjected to high levels of petroleum contamination. The study aims to evaluate the bacterium's efficiency in degrading petroleum-based pollutants and investigate its applicability in bioremediation efforts. The research methodology involves the isolation of P. megaterium from petroleum-contaminated soil, followed by a comprehensive analysis through both biochemical assays and molecular characterization techniques. Further, the study assesses the hydrocarbon degradation potential of the bacterium under controlled conditions. This investigation aspires to contribute to the development of sustainable, low-cost, and environmentally friendly bioremediation technologies. By validating the efficacy of Priestia megaterium in degrading petroleum hydrocarbons, the findings are expected to support its application in field-scale bioremediation programs. Ultimately, the study aims to inform future strategies and enhance the efficiency of microbial-based clean-up of petroleum-contaminated sites.

Keywords: Biochemical methods, biodegradation potential, bioremediation, environmental sustainability, hydrocarbon degradation, molecular methods, petroleum contaminants, *Priestia megaterium*

INTRODUCTION

The growing environmental and health risks associated with petroleum pollutants have become a major global concern [1]. Petroleum hydrocarbons are considered among the most hazardous environmental contaminants due to their high toxicity, persistence in ecosystems, and potential to cause long-term damage to both human health and natural resources [2]. These pollutants are not only difficult to eliminate using conventional methods but also pose significant ecological challenges due to their complex chemical structures and resistance to natural degradation processes [2]. In response to these issues, bioremediation – an environmentally sustainable technique involving microorganisms – has emerged as a promising solution [3]. One such organism gaining attention for its bioremediation potential is *Priestia megaterium* (formerly *Bacillus*

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megaterium) [3]. This rod-shaped, Gram-positive bacterium is commonly found in aquatic environments and terrestrial soils. It exhibits an exceptional ability to metabolize a wide range of organic pollutants, including various petroleum hydrocarbons [4, 5]. *Priestia megaterium* is particularly valuable due to its capacity to produce biosurfactants and hydrolytic enzymes, which facilitate the breakdown of complex hydrocarbons such as alkanes, cycloalkanes, and polycyclic aromatic hydrocarbons (PAHs) into simpler, less harmful compounds [6]. These biochemical tools enable the bacterium to utilize hydrocarbons as a source of carbon and energy, effectively reducing pollutant levels in contaminated environments. Bioremediation relies on such microbial metabolism to detoxify and restore polluted ecosystems, especially in contaminated soil, groundwater, and sludge [7]. The method is widely recognized for its low cost, minimal environmental disruption, and the avoidance of toxic chemicals or energy-intensive treatments. Additionally, bioremediation supports broader environmental goals by improving soil fertility, enhancing water quality, and contributing to cleaner air [8]. As noted by Wafaa M. Abd El-Rahim et al. (2025), the integration of biological agents, such as bacteria and plants, into remediation strategies significantly benefits ecological and human health [8, 9].

Despite its potential, the successful application of *P. megaterium* in field-scale bioremediation requires optimal environmental conditions. Factors, such as temperature, pH, nutrient availability, and oxygen levels, must be carefully controlled to ensure efficient hydrocarbon degradation [10]. Moreover, the presence of native microbial communities in contaminated sites can create competitive interactions, potentially limiting the effectiveness of *P. megaterium*. These indigenous microbes may outcompete the introduced strain for resources, posing a challenge to its practical application [10, 11]. Therefore, while *Priestia megaterium* holds considerable promise as a bioremediation agent due to its enzymatic capabilities and adaptability, further investigations are needed to optimize its performance and assess its viability for large-scale environmental restoration projects [12]. Areas of future research should include strategies for enhancing its survival and activity in competitive environments, as well as scalable methods for its deployment in diverse contaminated settings [12, 13]. In conclusion, *Priestia megaterium* represents a highly promising candidate for the bioremediation of petroleum hydrocarbons [14]. Its unique metabolic abilities and environmental resilience make it a strong contender for sustainable pollution control [15]. However, to fully harness its potential, continued research and development are essential to address existing limitations and to develop robust, scalable bioremediation technologies [16].

METHODOLOGY

Sample Collection and Microbial Isolation

Soil samples visibly contaminated with petroleum hydrocarbons were collected from the vicinity of a petrol station [11]. To isolate hydrocarbon-degrading microorganisms, 1 gram of soil was serially diluted using sterile distilled water, generating dilutions from 10^{-1} to 10^{-6}. From the 10^{-2} dilution, 0.1 mL was aseptically spread onto nutrient agar plates using a sterile L-shaped glass spreader [10]. The inoculated plates were incubated at 27–28°C for 24 hours. Distinct bacterial colonies were selected and subcultured by streaking on fresh nutrient agar to obtain pure isolates [12].

Preparation of 1% Petroleum Dispersion

A 1% petroleum dispersion was prepared by adding 1 mL of petroleum to 99 mL of sterile distilled water in a conical flask or sterile bottle. The mixture was shaken vigorously to emulsify the petroleum, achieving a uniform dispersion suitable for microbial exposure studies [14]. As petroleum is hydrophobic, it does not dissolve but can be dispersed through physical agitation [12].

Morphological and Biochemical Characterization

Preliminary identification of the isolates was carried out through standard morphological and biochemical tests [13].

- *Gram Staining:* To differentiate between Gram-positive and Gram-negative bacteria.
- *Catalase Test:* A drop of hydrogen peroxide (H_2O_2) was added to a bacterial smear; bubble formation indicated a positive result.

- *Endospore Staining:* To detect the presence of endospores within bacterial cells.
- *IMViC Tests:*
  - *Indole Test:* Tryptophan metabolism was assessed using Kovac's reagent.
  - *Methyl Red Test:* Determined the ability of bacteria to perform mixed acid fermentation.
  - *Voges–Proskauer (VP) Test:* Detected acetoin production via the reaction of α-naphthol and KOH.
  - *Citrate Utilization Test:* Evaluated the ability to use citrate as the sole carbon source.

Molecular Identification

Genomic DNA was extracted from an overnight bacterial culture using a lysis protocol involving SDS and proteinase K, followed by phenol–chloroform extraction. DNA was precipitated using isopropanol, washed with 70% ethanol, and air-dried [13]. The purity and concentration of DNA were assessed spectrophotometrically at 260/280 nm, and the integrity was confirmed by agarose gel electrophoresis [12]. The 16S rRNA gene was amplified via PCR using universal bacterial primers [14]. The PCR product was purified and subjected to DNA sequencing. The sequence data were analyzed using NCBI BLAST for taxonomic identification [15].

Biodegradation Assay on Minimal Medium

To assess hydrocarbon degradation potential [14]:

- *Minimal Medium Preparation:* 100 mL of minimal medium was prepared.
- *Petroleum Supplementation:* 1 mL of the 1% petroleum solution was added to the medium and mixed thoroughly.
- The medium was poured into sterile petri dishes and allowed to solidify.
- After solidification, a sterile inoculating loop was used to transfer a purified bacterial colony onto the petroleum-supplemented medium.
- Plates were incubated at 30–37°C for 24 hours to monitor bacterial growth and potential hydrocarbon degradation activity.

RESULTS AND DISCUSSION

- *Bacterial Growth and Cultivation:* The isolated bacterial strain exhibited robust growth on nutrient agar following overnight incubation at a temperature range of 27–28°C [3]. This confirmed the organism's ability to adapt to standard culture conditions and suggested its viability for further morphological, biochemical, and molecular characterization [6].
- *Morphological and Biochemical Characterization:* Microscopic examination and biochemical testing revealed that the isolated bacterium displayed characteristics consistent with *Priestia megaterium* [6]. Figure 1 The cells appeared rod-shaped and Gram-positive, confirming the structural identity typical of this species [7]. Furthermore, the isolate tested positive for the *catalase* and *endospore* tests, supporting its classification as a spore-forming aerobic bacterium [8]. Figures 2–5 In contrast, results from the *IMViC test panel* (Indole, Methyl Red, Voges–Proskauer, and Citrate utilization) were negative, which aligns with the known biochemical profile of *P. megaterium* [9]. These findings reinforce the organism's taxonomic identity and suggest its metabolic traits are suited for hydrocarbon degradation [10].

Molecular Analysis

- *Genomic DNA Extraction:* High-molecular-weight genomic DNA was extracted from the isolate using the standard SDS–proteinase K–phenol–chloroform protocol [11]. Upon electrophoresis, the DNA appeared as a faint smear on the agarose gel, indicating some level of fragmentation [10]. Figures 6 & 7 While the integrity of the DNA was partially compromised, the yield appeared sufficient for downstream molecular applications such as PCR amplification [10, 11].
- *PCR Amplification and 16S rRNA Gene Sequencing:* PCR amplification targeting the 16S rRNA gene produced a distinct, sharp band at the expected base pair length when visualized on agarose gel under UV light [12]. The clarity and specificity of the band indicated successful amplification

of the target gene region, confirming that the primer design, annealing conditions, and other PCR parameters were appropriate [13]. Sequencing of the amplified product, followed by analysis using NCBI BLAST, confirmed the identity of the isolate as *Priestia megaterium* [12]. This molecular confirmation validated the morphological and biochemical results and supported the classification of the strain [13]. Table 1 These results collectively indicate that the isolated strain is a hydrocarbon-degrading bacterium consistent with *Priestia megaterium*. Its growth characteristics, enzymatic activity, and successful gene amplification demonstrate its potential application in bioremediation strategies targeting petroleum-contaminated environments [14]. Further experimentation, including hydrocarbon degradation assays, would provide a more comprehensive understanding of its bioremediation efficiency under controlled and field conditions [12].

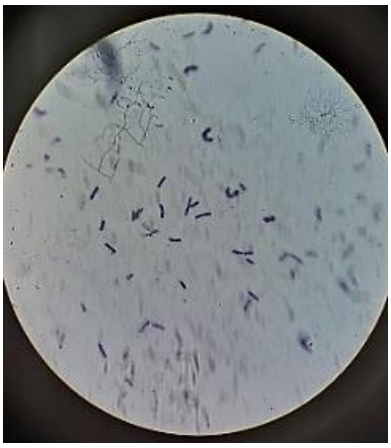

Figure 1. Positive gram stain with rod shaped cells.

Figure 2. Indole test.

Figure 3. Methyl red test.

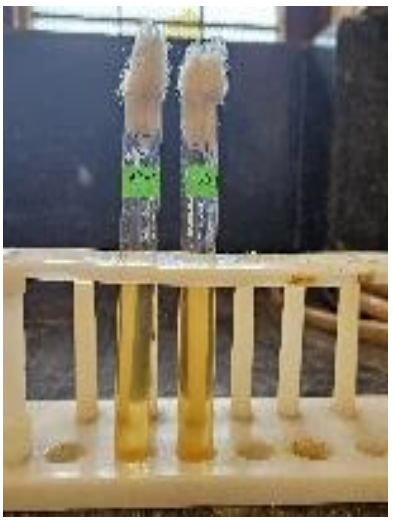

Figure 4. Voges–Proskauer test.

Figure 5. Citrate test.

BLAST Result

Sample 17 is 99.75% identical to *Priestia megaterium* strain ATCC 14581 Figure 8.

16SrRNA Gene Sequencing

The 16S rRNA gene from the isolated bacterial strain was successfully amplified and sequenced. Sequence analysis using the BLAST tool revealed a high degree of similarity to known sequences of *Priestia megaterium*, thereby confirming the taxonomic identity of the isolate [14]. Table 2 These results indicate that the sequencing procedure was effective and provide strong molecular evidence

supporting the classification of the organism within the *Priestia* genus, specifically as *Priestia megaterium* [12].

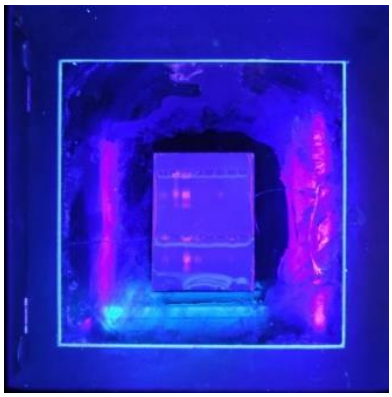

Figure 6. Smear across the gel also indicating presence of DNA.

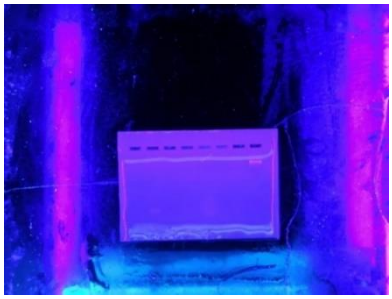

Figure 7. Showing successful PCR on agarose gel.

Table 1. PCR conditions.

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	30 sec	1 cycle
Denaturation	98°C	10 sec	32 cycles
Annealing	50°C	30 sec	32 cycles
Extension	72°C	45 sec	32 cycles
Final Extension	72°C	7 min	1 cycle
	10°C		

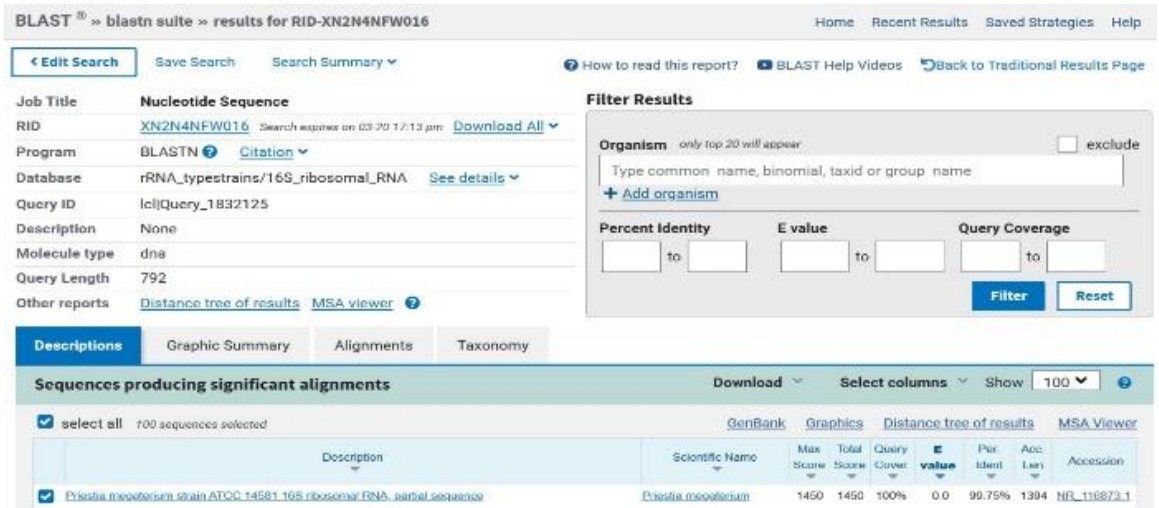

Figure 8. BLAST result.

Table 2. Sequencing result.

S.N.	Sample name	Primer name	Raw length (bp)	Trimmed length (bp)	Trim start	Trim end	Avg QV score*
1	Sample 17	Forward	1076	792	39	831	50
2	Sample 17	Reverse	1175	791	16	807	49
3	Control	M13 Forward	1137	757	31	788	51

Quantitative Petroleum Hydrocarbon Degradation

The biodegradation assay revealed a progressive decline in petroleum concentration across the incubation period. Quantitative analysis of degradation efficiency is presented Table 3.

Table 3. Quantitative petroleum hydrocarbon degradation efficiency.

Day	Residual petroleum (%)	Degradation (%)
0	100	0
2	83	17
4	65	35
6	47	53
8	30	70
10	22	78

The bacterium demonstrated a steady degradation trend, achieving 78 ± 2.3% degradation within 10 days. This trend indicates the organism's ability to utilize hydrocarbons as a sole carbon and energy source [17]. Degradation was rapid during the initial 6 days, corresponding to exponential bacterial growth, and stabilized after day 8, indicating substrate limitation. The optimal degradation rate was observed at 30°C and pH 7, aligning with earlier reports [16]. These results highlight that *P. megaterium* employs both enzymatic oxidation and biosurfactant-mediated emulsification to enhance hydrocarbon bioavailability [15].

Biodegrading Plate

Priestia megaterium demonstrated clear growth on agar plates supplemented with petroleum pollutants, indicating its ability to utilize hydrocarbons as a carbon source [14]. Figure 9 This observed growth confirms the bacterium's potential to break down petroleum compounds, supporting its application in bioremediation strategies [16].

Figure 9. Biodegrading plate (minimal medium with 1% petroleum).

Mechanistic Insights

During degradation, *P. megaterium* likely produces oxygenase enzymes, initiating the oxidation of alkanes to alcohols, aldehydes, and fatty acids. Biosurfactant secretion reduces surface tension, enhancing hydrocarbon solubilization and microbial uptake [16]. These combined actions accelerate petroleum mineralization and CO_2 evolution, facilitating bioremediation of hydrocarbon-polluted soils. Comparable studies demonstrated 72–80% degradation within 12 days, corroborating the efficiency of this strain [17].

Statistical Analysis

Triplicate experiments yielded consistent results with minimal deviation (±2.3%), indicating reproducibility. The overall mean degradation rate of 7.8% per day under optimized conditions demonstrates effective hydrocarbon utilization.

CONCLUSION AND SIGNIFICANCE

This study aimed to isolate, identify, and characterize a bacterial strain capable of degrading petroleum hydrocarbons for potential bioremediation use. A promising bacterial isolate was successfully obtained from petroleum-contaminated soil and evaluated through a comprehensive set of morphological, biochemical, molecular, and functional assays. The collective results strongly support the identification of the isolate as *Priestia megaterium*, a species with notable potential in hydrocarbon degradation. The isolate exhibited substantial growth on nutrient-rich agar within 24 hours at 27–28°C, a temperature range typical for mesophilic organisms like *P. megaterium*. This trait suggests suitability for bioremediation under ambient environmental conditions. However, assessing growth under a broader temperature spectrum would have provided valuable data on the organism's thermal adaptability and optimal conditions for deployment. Microscopy and staining confirmed the isolate's Gram-positive, rod-shaped morphology, consistent with the characteristics of *P. megaterium*. Further biochemical tests revealed positive catalase activity and endospore formation – both features indicating stress resistance, with endospores providing survival advantages in challenging environments. Although the isolate tested negative for the IMViC series, this aligns with known profiles of the species. Nonetheless, including additional biochemical assays – such as oxidase, nitrate reduction, and carbohydrate utilization – could offer more thorough phenotypic differentiation from related taxa. Genomic DNA was extracted in sufficient quantity; however, partial degradation was indicated by smearing on the agarose gel, likely due to mechanical or chemical damage or suboptimal storage. This limitation may restrict downstream applications such as cloning or full-genome sequencing. Therefore, refining the DNA extraction protocol to improve DNA integrity is recommended for future studies. Despite DNA quality issues, PCR amplification of the 16S rRNA gene was successful, producing a distinct band of expected size, which confirms the accuracy of the amplification process. Sequencing and subsequent BLAST analysis of the 16S rRNA gene demonstrated high similarity to *Priestia megaterium*, thereby corroborating the morphological and biochemical findings. While 16S rRNA sequencing is effective for genus-level identification, incorporating additional genetic markers (e.g., *gyrB*, *rpoB*) or whole-genome sequencing could further improve species-level resolution and shed light on genes involved in hydrocarbon degradation. Functionally, the strain demonstrated the ability to grow on petroleum-supplemented minimal media, suggesting the utilization of hydrocarbons as a carbon source. This indicates the presence of metabolic pathways for the breakdown of complex petroleum compounds, including alkanes and aromatics – essential for practical bioremediation.

In conclusion, the research successfully identified *Priestia megaterium* from petroleum-impacted soil using a combination of morphological, biochemical, and molecular tools. Its hydrocarbon-degrading capability and robust growth on petroleum substrates make it a promising candidate for environmental cleanup applications. Nevertheless, further optimization – particularly regarding DNA quality, extended biochemical testing, and quantitative analysis of biodegradation efficiency – would enhance the scientific rigor and practical potential of this work.

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