RESEARCH ARTICLE

Exploring the Polyethylene Degradation Efficiency of *Bacillus vallismortis* SK070

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Abstract

In the rapidly growing world, plastic consumption has increased on a large scale, intensifying environmental pollution. As conventional waste management approaches prove inadequate, microbial degradation emerges as a promising and innovative solution. This study investigates the microbial communities in soil contaminated with plastic waste from a dump yard in Jawahar Nagar, Hyderabad, Telangana. A composite soil sample, SKP007, was analyzed using 16S V3-V4 region metagenomic sequencing on the Illumina MiSeq platform. Phylum-level analysis revealed predominant groups, including *Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria* and *Firmicutes*, with *Bacillus*, *Rubrobacter*, *Streptomyces*, and *Steroidobacter* as dominant genera. Following a 14-day enrichment period, six bacterial isolates were screened for plastic degradation. Isolate SK070 exhibited the highest plastic degradation efficiency, with a clearance zone diameter of 4.2 ± 0.3 mm and a degradation rate of 3.95 ± 0.06%. Morphological characterization of SK070 revealed rod-shaped bacteria with blunt ends. Molecular analysis through 16S rRNA sequencing identified SK070 as *Bacillus vallismortis*, showing 99.99% similarity. Phylogenetic analysis confirmed its close relationship with *Bacillus subtilis*. This study highlights *B. vallismortis* SK070 as a promising candidate for plastic bioremediation, warranting further investigation into its optimization and genomic features.

Highlights

- The present study elucidates the involvement of microorganisms in the degradation of polyethylene materials.
- 16S rRNA V3-V4 region metagenomic sequencing reveals the microbial diversity within the soil sample.
- The investigation demonstrates the potential of the SK070 strain for plastic degradation.
- Morphological and molecular characterization of SK070 identifies the bacterium as *Bacillus vallismortis*.

Keywords: *Bacillus vallismortis*, Metagenome, Plastic-degradation

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Introduction

Rapid urbanization and industrialization have led to the excessive utilization of plastic materials, which accumulate as plastic waste, posing significant environmental concerns. Over the past 50 years, advancements in technology have drastically increased plastic usage, with current projections suggesting that plastic production will triple by 2050, rising from 350 million tonnes to 1,100 million tonnes globally (Dang *et al.,* 2018). Approximately 50% of this plastic is inadequately managed, contributing to environmental pollution (Veiga *et al.,* 2016). This plastic waste often accumulates in rivers, water bodies, and coastal regions, leading to the formation of microplastics (Ozdemir *et al.,* 2022). Plastics are long-chain hydrocarbon polymers derived from petrochemicals, synthesized to form high molecular weight polymers (Nedi *et al.,* 2024). They primarily consist of hydrogen, oxygen, chloride, nitrogen, carbon, and silicon, with polyethylene (C_nH_{2n}) representing 64% of the total plastic materials (Zeenat *et al.,* 2021). The synthetic plastic industry has experienced exponential growth, with production increasing twenty-fold since 1964. Plastics are favored for their lightweight, durability, and strength (Yang *et al.,* 2020). However, their environmental impact is significant, as they take a long time to degrade and accumulate in large quantities (Al-Thawadi, 2020).

Traditional methods for managing plastic waste, such as recycling, incineration, and landfilling, also present environmental challenges, including toxic gas emissions, high costs, and prolonged degradation times. An eco-friendly approach employing microbial degradation presents a promising alternative for addressing plastic accumulation (Hadad *et al.,* 2005). Plastics are broadly categorized into thermosets and thermoplastics. Thermoplastics can be melted and molded upon heating, while thermosets cannot (Aamer *et al.,* 2008). Key plastics include polypropylene, polyethylene, polyvinyl chloride, polyurethane, polystyrene, and polyethylene terephthalate, with polyethylene being a major environmental pollutant, accumulating 25 million tonnes annually worldwide (Ali *et al.,* 2021). Polyethylene is characterized by its lightweight, resistance, durability, flexibility, non-corrosiveness, and chemical resistance. However, its excessive production and accumulation pose threats to both human health and the environment by releasing toxic gases such as dioxins, nitrogen oxides, carbon monoxide, hydrogen cyanide, and dioxins. Furthermore,

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accumulated plastic can enter the food chain and be transferred to higher trophic levels (Munir *et al.,* 2018).

Various microbes, including *Pseudomonas* spp., *Aspergillus* spp., *Arthrobacter* spp., *Rhodococcus* spp., *Alacnivox* spp., *Achromobacter* spp., *Bacillus* spp., *Enterobacter* spp., *Serratia* spp., *Zalerion* spp., and *Phormidium* spp., have been identified for their role in polyethylene degradation (Nedi *et al.,* 2024). Among these, *Bacillus* spp. is notable for its rapid growth and endospore formation, allowing it to thrive in diverse and harsh environmental conditions (Karuganti *et al.,* 2023; Sukumar *et al.,* 2023). Typically, linear alkanes of polyethylene are expected to be biodegradable; however, the biodegradability of polyethylene inversely correlates with its molecular weight. Microbes can utilize linear chains of hydrocarbon oligomers with a molecular weight of less than 620, but the high molecular weight chains of polyethylene resist biodegradation (Hadad *et al.,* 2005).

Biodegradation occurs when microbes use polyethylene as a carbon source, breaking down the polymer. This process can be accelerated if microbes form biofilms on the polyethylene surface. However, the hydrophobic nature of polyethylene impedes biofilm formation. Microbes secrete enzymes that depolymerize polyethylene into monomers that are less toxic to the environment and humans. Specific genes, such as *alkB* in *Pseudomonas aeruginosa*, encode enzymes responsible for polyethylene degradation (Skariyachan *et al.,* 2015).

This study introduces a novel approach by isolating and characterizing a *Bacillus sp*. capable of efficiently degrading polyethylene at elevated temperatures. Unlike mesophilic bacteria, *Bacillus* spp., exhibit enhanced enzymatic activity and biofilm formation, accelerating polyethylene breakdown. Additionally, their heat-resistant endospores enable survival in harsh environments, making them ideal for waste management applications. This research advances bioremediation by identifying a high-efficiency strain and evaluating its degradation potential, offering a sustainable and eco-friendly solution for managing plastic waste.

Materials and Methods

Samples collection

Soil adhered to the plastics was collected in sterile gamma-irradiated containers. 500 g of each sample was collected from the garbage dumping yard of Jawahar Nagar, Hyderabad, Telangana (17.4426° N, 78.4303° E). Soil samples were specifically collected from a depth of 10-20 cm. A total of 20 samples were collected from different sites, homogenized, pooled as one sample and stored in the refrigerator at 4°C for further studies (Karuganti *et al.,* 2023; Raju *et al.,* 2023). These samples were transported to the AADHAAR, R&D Laboratory of Biofac Inputs Private Limited in Hyderabad and combined into a composite sample, designated SKP007.

Determination of microbial abundance

DNA was extracted from the composite soil sample and subjected to 16S V3-V4 region metagenomic sequencing using the Illumina MiSeq platform. Quality assessment of the DNA sequences was carried out using FastQC and MultiQC software tools. The diversified V3 – V4 region of 16S rRNA was amplified using universal primers. 341 F and 785 R with wide coverage were used for V3-V4 region because of its high hypervariable and heterogenic nature. The 16S rRNA V3-V4 region was amplified using Illumina Miseq and V3V4F: 5′ CCTACGGGNGGCWGCAG3′ V3V4R: 5′GACTACHVGGGTATCTAATCC3′ primers were used. Using P7 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA and P5 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT adapter sequences, the development of sequencing libraries was carried out. The low-quality sequences, adapter sequences and degenerate primers were trimmed using Trimgalore. The good-quality contigs were processed and merged to form the final sequence. The selected final sequence was distributed into individual taxonomical units using the GREENGENES database and microbial abundance was estimated (Raju *et al.,* 2023).

Isolation of polyethylene-degrading bacteria

About 10 g of soil sample was added to 90 mL of 0.85% (w/v) NaCl solution. A 10 mL of suspension was inoculated aseptically into 100 ml of selective medium with composition g/L 0.7 K_2HPO_4, 0.7 KH_2PO_4, 0.7 $MgSO_4$, 0.005 NaCl, 1 NH_4NO_3, 0.002 $FeSO_4$, 0.002 $ZnSO_4$, 0.001 $MnSO_4$, 1 polyethylene granules and kept on orbital shaker at 37°C for 14 days at 120 rpm. Plastic degrading microbial isolates were screened on the selective media with composition g/L 0.2 $CaCl_2$, 0.16 K_2HPO_4, 0.2 $MgSO_4$, 0.1 $(NH_4)_2SO_4$, 0.05 $MnSO_4$, 0.01 $FeSO_4$, 0.05 $ZnSO_4$, 0.01 $FeSO_4$, 15 agar, 1 polyethylene granules and incubated for 5 days at 37°C. Bacterial colonies showing zone of clearance were screened, purified, subcultured and stored at 4°C (Nedi *et al.,* 2024).

Polyethylene biodegradation assay

A polyethylene biodegradation assay was performed by taking 2x2 cm polyethylene pieces. Polyethylene pieces were exposed to UV radiation for 10 days in UV illumination hood. The polyethylene pieces were disinfected with 70% ethanol for 30 minutes, dried overnight and weighed. The polyethylene pieces were added to the flasks containing 100 mL of mineral salt medium containing g/L 1 K_2HPO_4, 1 NH_4NO_3, 0.2 $MgSO_4$, 0.1 $CaCl_2$, 0.15 KCl, 0.001 $ZnSO_4$, 0.001 $FeSO_4$, 0.001 $MnSO_4$. 4 ml of freshly grown plastic degrading isolate was inoculated in the flasks and incubated for 30 days at 30°C. The degraded plastic pieces were washed with 2% SDS (Sodium dodecyl sulfate) solution at 60°C for 4 hours, followed by distilled water wash. The treated plastic pieces were dried in a hot air oven at 60°C and the final weight loss was calculated as Weight loss % = (Initial weight – Final weight/Initial weight)x100 (Sangeetha Devi *et al.,* 2015).

Characterization of the polyethylene degrading bacteria

An efficient polyethylene degrading bacteria was morphologically studied using the scanning electron microscope Hitachi S-3700 model. Molecular characterization was done by using 16S rRNA gene sequencing using Sanger's method. The 16S rRNA gene was amplified using 518 F- CCAGCAGCCGCGGTAATACG and 800 R – TACCAGGGTATCTAATCC primers (Busi *et al.,* 2017; Karuganti *et al.,* 2023). 16S rRNA sequencing was carried out in Apical Scientific Sdn Bhd, Malaysia. Raw DNA sequences were checked for incomplete data, blank spaces and chimeric

sequences using Chromapro 2.0. The adapter and chimeric sequences were trimmed and the DNA sequence data was queried in NCBI BLAST. DNA sequences were extracted from the NCBI BLAST and CLUSTAL W was used to align the sequences. To understand the evolutionary history, a neighbour joining tree with a distance-based method was constructed using MEGA XI and the maximum composite likelihood method was used to assess the evolutionary distance (Ozdemir *et al.,* 2022).

Results and Discussion

Determination of microbial abundance

Metagenomic sequencing generated a total of 101,016 reads, with an average read length of 301 base pairs (bp). The GC content of the sequences was determined to be 57.5%. Quality metrics indicated that 99.69% of the reads had a quality score greater than Q20, and 90.63% of the reads had a quality score greater than Q30.

Metagenomic data analysis at the phylum level identified several predominant phyla, including *Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria* and *Firmicutes*. At the genus level, the dominant genera were *Bacillus, Rubrobacter, Streptomyces* and *Steroidobacter*. The high prevalence of the Bacillus genus is likely due to its rapid growth rate, endospore formation, and adaptability to harsh environmental conditions (Fig. 1).

The metagenomic analysis of soil samples provided insights into the microbial diversity present in environments contaminated with plastics. The predominant phyla identified were *Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria* and *Firmicutes,* which were consistent with findings from similar studies on contaminated soils. For instance, *Proteobacteria* and *Firmicutes* were frequently reported as dominant phyla in plastic-contaminated soils due to their adaptability and potential roles in organic matter degradation (Reddy *et al.,* 2023; Shilpa *et al.,* 2022; Purohit *et al.,* 2020).

The genus-level identification revealed a high abundance of *Bacillus*, *Rubrobacter*, *Streptomyces*, and *Steroidobacter*. The dominance of *Bacillus* is particularly noteworthy. *Bacillus* species are known for their robust metabolic capabilities and resilience to environmental stressors, including exposure to pollutants (Kumar *et al.,* 2021). Their presence in high abundance in plastic-contaminated soils supports their potential role in bioremediation.

Screening of plastic degrading isolates

Following a 14-day enrichment period in selective media, the soil sample was screened to isolate efficient plastic-degrading organisms. Six isolates were identified based on the formation of clearance zones and were designated SK010, SK040, SK070, SK080, SK090, and SK110. The efficacy of plastic degradation was assessed by measuring the diameter of the clearance zones formed around each isolate. Among the isolates, SK070 demonstrated the highest efficiency, with a clearance zone diameter of 4.2 ± 0.3 mm. This was followed by SK040, which produced a clearance zone with a diameter of 3.8 ± 0.2 mm (Fig. 2).

After a 30-days of incubation, the percentage of plastic degradation was calculated for each isolate using the formula weight loss

% = (Initial weight – Final weight/Initial weight) x 100

Among the isolates, SK070 demonstrated the highest plastic degradation capability, with a degradation rate of 3.95 ± 0.06%. This was followed by SK040, which achieved a degradation rate of 3.21 ± 0.05% within a span of 30 days. In contrast, SK090 exhibited the lowest plastic degradation, with a rate of 0.87 ± 0.03% (Fig. 3).

The isolation and assessment of plastic-degrading capabilities of six bacterial isolates from the contaminated soil revealed that SK070 had the highest efficiency in both clearance zone diameter and polyethylene degradation rate. SK070's clearance zone diameter of 4.2 ± 0.3 mm and a degradation rate of 3.95 ± 0.06% after 30 days were the highest among the tested isolates.

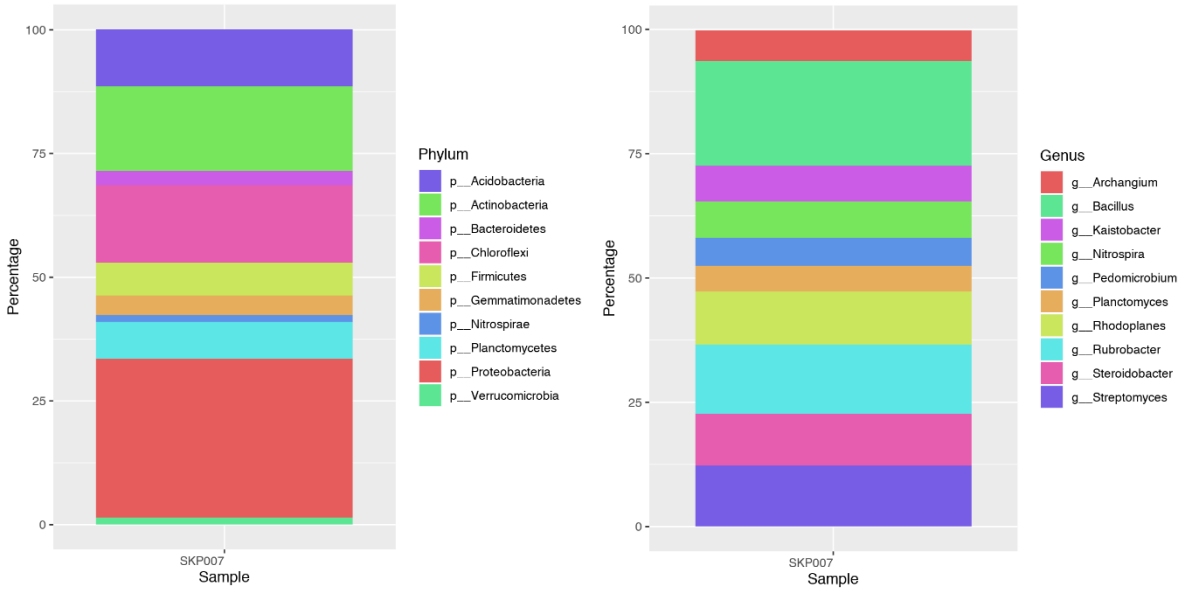

Fig. 1: Stacking bar graph showing on the left: phylum abundance, on the right: genus abundance

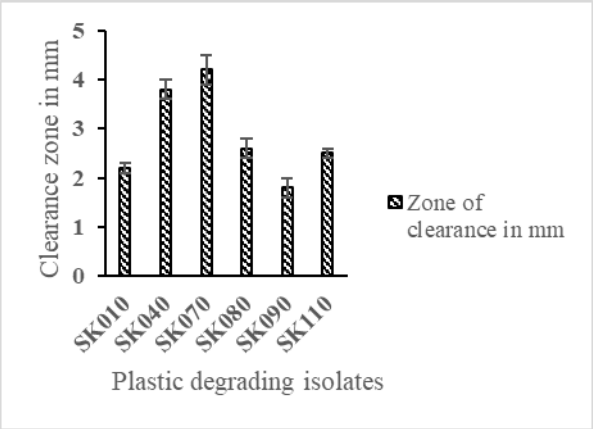

Fig. 2: Graph illustrating the degradation of plastic and the formation of clearance zones by plastic-degrading bacteria

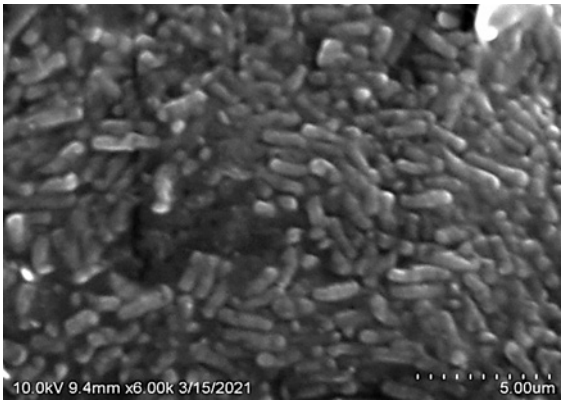

Fig. 4: Scanning electron microscope image at 10000X magnification displaying the structural details of SK070

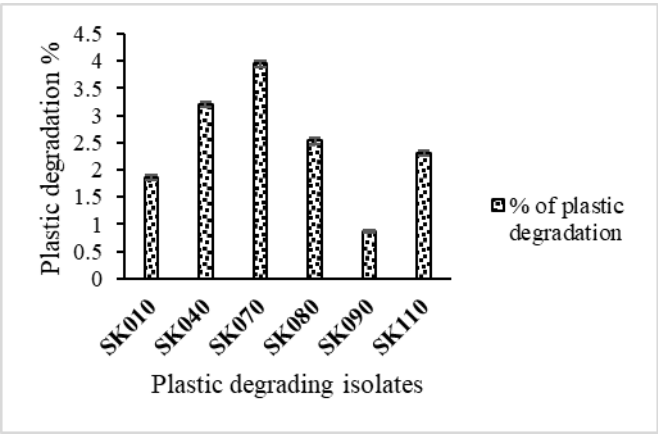

Fig. 3: Graph demonstrating the plastic degradation capability of microbial isolates

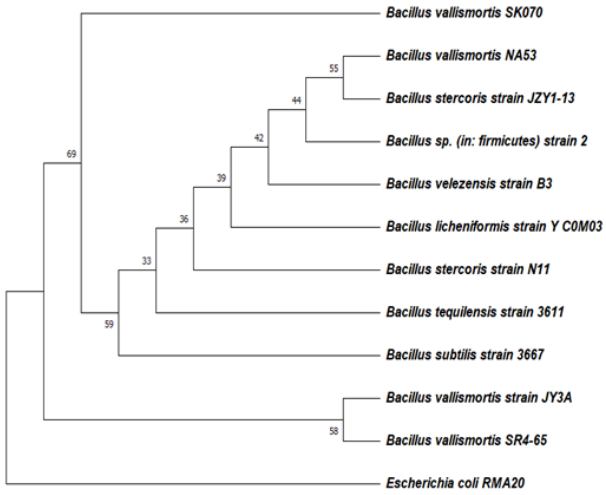

Fig. 5: Neighbor-joining phylogenetic tree of SK070 Strain: Demonstrating the close phylogenetic relationship with *B. vallismortis* constructed using 1000 boot strap replications

These findings align with previous research that has documented the ability of *Bacillus* species to degrade plastics. For example, *Anoxybacillus* spp. Showed high efficiency in breaking down plastic materials within 40 days (Ozdemir *et al.,* 2022). Similar results were reciprocated in *Paenibacillus amylolyticus* (Teeraphatpornchai *et al.,* 2003). The results obtained are in par with the results of *Bacillus subtilis* and *Bacillus licheinformis* with weight losses of 3.49% and 2.83% within 30 days (Yao *et al.,* 2022). The higher degradation efficiency of SK070 may be attributed to specific enzymes or metabolic pathways unique to this strain, which facilitate the breakdown of polyethylene more effectively.

The comparative analysis also highlighted SK040 as the second most effective isolate, with a degradation rate of 3.21 ± 0.05%. In contrast, SK090 showed a much lower degradation rate (0.87 ± 0.03%), indicating variability in the plastic-degrading capabilities among different bacterial isolates, which is a common observation in bioremediation studies.

Characterization of the polyethylene degrading bacteria

SK070 isolates showing a high zone of plastic degradation were characterized using morphological and molecular approaches. Scanning electron microscopic observation at 10000 X revealed the shape of the bacteria as rod-shaped bacteria with blunt ends (Fig. 4).

The SK070 strain was characterized through 16S rRNA sequencing. Raw DNA sequences were initially trimmed using ChromasPro 2.0 to ensure quality. High-quality sequences were subjected to a BLAST search in the NCBI database, which revealed a 99.99% similarity with *Bacillus vallismortis*. The sequence data for SK070 was subsequently deposited in the NCBI GenBank under accession number MT093348. Phylogenetic analysis was conducted using MEGA XI software, with a Neighbor-Joining (NJ) tree constructed based on 1000 bootstrap replications. Reference DNA sequences were retrieved from the NCBI database for comparative analysis. *Escherichia coli* was used as an outgroup. The phylogenetic tree demonstrated that *B. vallismortis* SK070 exhibits a close phylogenetic relationship with *B. vallismortis* and *Bacillus subtilis* (Fig. 5).

The morphological characterization of SK070 as rod-shaped bacteria with blunt ends, observed through scanning electron

microscopy, aligns with the typical morphology of *Bacillus* species (Chakraborty *et al.,* 2014). Molecular characterization using 16S rRNA sequencing identified SK070 as *B. vallismortis*, showing a 99.99% similarity with known strains of this species. Phylogenetic analysis confirmed its close relationship with *Bacillus subtilis*, a well-known plastic-degrading bacterium (Vimala and Mathew 2016).

The identification of SK070 as *B. vallismortis* is significant because this species has been less studied in the context of plastic degradation. The close phylogenetic relationship with *Bacillus subtilis* suggests that SK070 may possess similar plastic-degrading mechanisms, potentially involving extracellular enzymes or metabolic pathways shared with other *Bacillus* species (Ali *et al.,* 2021).

The results from this study contribute to the growing body of knowledge on microbial plastic degradation. The high plastic degradation efficiency of SK070 is comparable to other efficient plastic-degrading strains reported in the literature. The lower degradation rate of SK090 underscores the variability in plastic-degrading capabilities among different bacterial strains. This variability highlights the importance of comprehensive screening and optimization in identifying and enhancing plastic-degrading microorganisms. The differences observed in plastic degradation rates among isolates, including SK070's superior performance, reinforce the need for further research into the specific enzymes and metabolic pathways involved in plastic degradation (Reddy et al., 2023; Yao *et al.,* 2022).

Conclusion

The current study investigates the microbial community in a plastic dump yard, identifying the predominant phyla as *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Acidobacteria*, and *Firmicutes*. Screening of soil samples from this site led to the isolation of a rapid plastic-degrading bacterium, SK070, which was subsequently characterized as *B. vallismortis*. The results indicate that *B. vallismortis* SK070 is highly effective in plastic degradation. Future research should focus on optimizing the degradation conditions and elucidating the genomic underpinnings of this strain to enhance its bioremediation potential.

Author Contributions

All the authors contributed equally to experiments, preparation of the manuscript, and reviewing and editing of the initial draft. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

Authors have no conflict of interests.

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