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Shotgun Metagenomic Profiling Of Polyethylene-Degrading Microbiomes In Indian Landfills

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ABSTRACT

Polyethylene (PE) waste in municipal landfills is a major pollutant, yet the indigenous microbial consortia capable of its degradation are poorly characterized. In this study, we performed a comprehensive shotgun metagenomic analysis of microbial communities from three Indian landfill sites (Okhla, Ghazipur, and Pirana) using publicly available sequence datasets (SRA/ENA). Reads were quality-controlled (Trimmomatic, FastQC) and taxonomically profiled (Kraken2); taxa of interest were cross-referenced against NCBI, BacDive, MBGD, and Ensembl Bacteria to identify known and putative PE-degrading bacteria. To our knowledge, this is the first study explicitly exploring PE-degradation potential via metagenomics at the Okhla and Ghazipur landfills. We found that the Okhla landfill community was dominated by Actinobacteria (~48.5% of bacterial reads), notably *Streptomyces* and *Saccharomonospora*. Across sites, several genera previously linked to polymer degradation were detected, including *Saccharomonospora*, *Streptomyces* (Actinobacteria), *Rhodococcus* (particularly *R. erythropolis*), and *Cupriavidus* (e.g. *C. campinensis*). Overall, the prevalence of these taxa and enzymes suggests that Indian landfill microbiomes harbor significant plastic-degrading capacity. These findings provide a foundation for bioremediation strategies and the discovery of novel plastic-degrading enzymes from landfill microbial communities

Keywords: polyethylene degradation, landfill microbiome, metagenomics, plastic pollution, Actinobacteria, *Streptomyces*, *Saccharomonospora*, hydrocarbon-degrading bacteria, microbial consortia, environmental biotechnology

1. INTRODUCTION

1.1 Global Plastic Pollution Crisis

The widespread use of plastics, driven by their versatility, durability, and low cost, has resulted in a significant global environmental burden. With annual production exceeding 150 million tonnes and consumption growing at roughly 5% per year, plastics have become among the most persistent pollutants in both terrestrial and marine ecosystems. Their resistance to degradation—owing to strong carbon-hydrogen bonds and stabilizing additives—allows them to persist for centuries, contributing to widespread contamination, including microplastic infiltration into food webs.

Beyond physical accumulation, plastics release toxic substances such as dioxins, phthalates, and heavy metals during degradation, posing health risks to humans and wildlife. Mitigation efforts like recycling and chemical treatments remain insufficient, particularly in regions with underdeveloped waste management systems. The COVID-19 pandemic further escalated the crisis through increased single-use plastic consumption, notably an estimated 3.5 million metric tonnes of discarded PPE between 2020 and 2021 (Patrício Silva et al., 2021).

Polyethylene (PE), derived from petrochemical ethylene, is among the most extensively produced and discarded plastics due to its broad industrial applications—especially in packaging, which accounts for 59% of India's plastic use (Sustainability, 2022). Its durability and economic viability make PE a critical target for sustainable intervention. Addressing its environmental impact necessitates not only enhanced waste management but also innovative biological degradation strategies. Plastic waste management in India is hindered by systemic inefficiencies in collection, segregation, and recycling. Although 80.28% of plastic waste is collected, only 28.4% is treated, largely due to contamination, inadequate sorting, and fragmented infrastructure (CPCB, 2020). The informal sector—critical to waste handling—remains unregulated, exposing workers to health risks and limiting integration into formal systems (Yang et al., 2017).

Key challenges include the scarcity of material recovery facilities (MRFs), low processing capacity for diverse plastic types, and insufficient incorporation of informal labor into the formal economy. Sectors reliant on PE, PP, and PVC—such as packaging and construction—are particularly affected (Bajaj et al., 2021). Although India’s Extended Producer Responsibility (EPR) policy was introduced in 2016 and amended in 2018, poor enforcement and lack of clarity impede its effectiveness (Pani & Pathak et al., 2020).

Urban and coastal recycling systems are constrained by high energy demands and transportation costs (Nabavi-Pelesaraei et al., 2020), while centralized facilities struggle with operational inefficiencies. Decentralized solutions like micro-factories offer promise, but downcycling remains prevalent, reducing the value of recycled products (Jain & Soni, 2020). Moreover, the unregulated import of plastic waste and inadequate tracking systems add pressure to domestic recycling efforts (Singh et al., 2022).

Coastal plastic leakage, insufficient technologies for sorting multilayer plastics, and the lack of advanced reprocessing infrastructure continue to challenge effective waste recovery (Rajagopal et al., 2019; Patil et al., 2021). Addressing these barriers requires standardized technologies, economic incentives, and robust legal frameworks to improve recycling efficiency and stakeholder participation (Schmaltz et al., 2020).

1.2 Microbial Plastic Degradation

The persistent accumulation of polyethylene (PE) in the environment has prompted the search for sustainable waste management alternatives. PE’s long carbon chains make it highly resistant to degradation, leading to long-term environmental contamination (Andrady, 2015). Microbial degradation, which involves the enzymatic breakdown of polymers by microorganisms, offers a potential solution, unlike mechanical recycling, which often results in downcycled products and environmental harm (Mohanan et al., 2020).

Certain microorganisms, including bacteria, fungi, and actinomycetes, have evolved enzymes such as alkB and laccases that can degrade PE by breaking its long carbon chains into simpler, metabolizable compounds (Shah et al., 2018). Advancements in metagenomics and molecular biology have deepened our understanding of these microbial degradation pathways, presenting new opportunities for mitigating plastic waste.

However, microbial degradation of PE is still in its infancy. The process is typically slow, and environmental conditions must be carefully controlled to optimize microbial activity (Fujita et al., 2016). Despite considerable research on microbial degradation, there is a lack of metagenomic studies specifically focused on PE-degrading microbes, particularly in regions like India, where plastic pollution is a critical issue (Prakash et al., 2022).

The slow rate of degradation and the need for further optimization of microbial strains and enzymatic pathways remain significant challenges. Research into PE-degrading microbes in specific contexts like Indian landfills is particularly underdeveloped, hindering the development of scalable biotechnological solutions for plastic waste management (Joshi et al., 2021).

1.3 Enzymatic Plastic Degradation

Polyethylene (PE) degradation is facilitated by specialized enzymes that break down its inert carbon-carbon bonds. Key enzymes involved in this process include alkane hydroxylases, such as alkB, and oxidoreductases like laccases. These enzymes initiate the breakdown of PE into smaller, biodegradable compounds. AlkB, in particular, is effective at degrading aliphatic hydrocarbons, a major component of PE, by converting them into alcohols that are more easily metabolized. Bacteria like Pseudomonas putida and Acinetobacter sp. have demonstrated the potential for PE degradation in laboratory conditions (Nakanishi et al., 2020).

Laccases, copper-containing enzymes, catalyze oxidation reactions that make PE more susceptible to microbial attack. Fungal species such as Trametes versicolor and Ganoderma lucidum have shown promise in breaking down PE through oxidative reactions (Rizvi et al., 2020; Hasan et al., 2021). However, current research continues to focus on identifying other novel enzymes, especially those that could function effectively under the unique conditions of environments like landfills, where factors such as low oxygen and high pressure present challenges.

These enzymes are crucial for developing biotechnological solutions to plastic waste management. Yet, many degradation pathways only partially break down PE, producing toxic or resistant byproducts. Identifying additional enzymes that can work together to enhance the breakdown process is essential for advancing PE degradation technologies.

Metagenomics presents a powerful, culture-independent strategy for identifying polyethylene (PE)-degrading microbes, particularly relevant in countries like India facing escalating plastic waste challenges. Unlike traditional culturing methods, metagenomics enables comprehensive profiling of microbial communities by analyzing total environmental DNA, thus revealing both known and previously uncultivable taxa involved in plastic degradation.

This approach facilitates the discovery of novel genes and enzymes capable of degrading PE under extreme landfill conditions, opening avenues for new biodegradation pathways (Singh et al., 2019). Moreover, metagenomic analysis accelerates the identification of microbial strains and consortia with high PE-degrading potential, bypassing the need for laborious isolation and culturing (Kumar et al., 2020). Such insights are crucial in India, where scalable, low-cost solutions are needed due to limited recycling infrastructure.

Importantly, metagenomics can uncover microbial adaptations to India’s unique landfill environments—characterized by high heat, humidity, and chemical complexity—thereby offering region-specific and globally applicable biotechnological strategies for plastic waste management (Vyas et al., 2019).

1.4 Study Rationale and Focus

Polyethylene (PE), including both low-density (LDPE) and high-density (HDPE) forms, is a highly persistent environmental pollutant due to its chemical inertness and biodegradation resistance. Conventional recycling methods are economically unsustainable, particularly in India, where the plastic waste issue is escalating. Microbial degradation offers a promising, eco-friendly alternative, utilizing natural metabolic pathways to break down PE into harmless compounds. However, most existing research has focused on isolated microbial strains, with limited community-level studies, and little attention has been given to landfills in India.

This study employs a metagenomic approach to explore the microbiomes of Indian landfills, aiming to identify microbial species and enzymatic systems capable of degrading polyethylene. By using metagenomics, the study overcomes the limitations of traditional culturing methods, enabling the discovery of both culturable and non-culturable microbes, and novel degradation pathways. Special focus will be given to enzymes like alkane monooxygenase (alkB) and laccases, known for their roles in PE breakdown through oxidative and depolymerization processes.

This research will be the first targeted metagenomic survey of PE-degrading microbial communities in Indian landfills, providing crucial insights into functional genes linked to plastic degradation. These findings will pave the way for future biotechnological innovations, such as enzyme engineering and microbial consortia development, to improve plastic waste management.

1.5 Research Objectives

* To apply metagenomic sequencing to characterize microbial communities in Indian landfill sites, focusing on polyethylene degradation potential.

* To determine the taxonomic composition of microbial populations associated with polyethylene degradation.

* To assess the diversity and abundance of degradation-associated genes across various landfill environments.

* To evaluate the biotechnological potential of identified microbes and enzymes for sustainable plastic waste management applications.

1.6 Research Questions

* What microbial taxa are present in Indian landfill sites with potential for polyethylene degradation?

* What are the implications of the identified microbial and enzymatic profiles for developing biotechnological solutions for plastic waste management?

2. METHODOLOGY

2.1 Data Retrieval

For this study, shotgun metagenomic datasets from municipal landfill sites in India were obtained from the Sequence Read Archive (SRA) and the European Nucleotide Archive (ENA), two widely used repositories for sequencing data. Three datasets were selected to capture microbial diversity across different landfill environments and geographical settings.

Dataset 1 was collected from the Okhla landfill in New Delhi (Accession: **SRR11086757**), representing microbial communities from leachate and soil samples. **Dataset 2**, sourced from the Ghazipur landfill in New Delhi (Accession: **SRX2861368**), focuses on microbial dynamics within layers of municipal solid waste. **Dataset 3** was obtained from the Pirana landfill in Gujarat (Accession: **ERR2040440**), one of the largest and oldest landfills in western India, offering insights into microbial communities shaped by extensive waste accumulation and anaerobic conditions.

All datasets were retrieved in **FASTQ format** using ENA’s FTP access for consistency and ease of processing. Associated metadata, including sample type and sequencing platform, was reviewed to ensure comparability across datasets before quality assessment and downstream analysis.

2.2 Preprocessing

Preprocessing was performed to remove technical artifacts and low-quality regions from raw metagenomic reads prior to analysis. **Trimmomatic** (Bolger et al., 2014) was used in **paired-end mode** for all datasets, applying a standardized sequence of quality control steps.

First, Illumina adapter sequences were removed using the *ILLUMINACLIP* function to prevent misalignments. Low-quality bases with Phred scores below 3 were trimmed from both the 5′ and 3′ ends of reads. A dynamic trimming step was then applied using a 4-base sliding window, cutting the read when the average quality within the window dropped below 20. Reads shorter than 36 bp after trimming were discarded, as they lack sufficient sequence length for accurate alignment or taxonomic classification.

These measures ensured the removal of sequencing noise, adapter contamination, and degraded regions, improving the reliability of downstream taxonomic profiling. Maintaining consistent preprocessing protocols across datasets minimized analytical bias and ensured data comparability. The integrity of paired-end read synchronization was confirmed before proceeding to quality assessment and taxonomic analysis.

These quality control measures were essential to minimize the chance of introducing sequencing biases into the taxonomic profiling. All preprocessing procedures were conducted in a reproducible and consistent way across datasets to maintain analytical uniformity. After trimming, the integrity of the dataset and read-pair synchronization were confirmed before moving on to quality assessment.

A diagram demonstrating the Trimmomatic workflow—showing how each of these processes interacts with paired-end data—can be inserted here for clarity.

2.3 Assessment of Raw Read Quality

Following the preprocessing phase, we conducted a quality assessment of both the raw and trimmed metagenomic reads using FastQC (Andrews, 2010), a tool commonly utilized for evaluating the quality of high-throughput sequencing data. This vital quality control step ensures that the reads used for subsequent taxonomic profiling are of adequate quality, devoid of sequencing artifacts, and representative of the microbial content in the environmental sample.

FastQC produces various diagnostic graphs and summary statistics that assess essential quality metrics across the sequencing reads. The tool offers quality scores per base and per sequence, along with information on adapter content, GC content, sequence length distribution, duplication levels, and overrepresented sequences. These metrics are instrumental in determining the effectiveness of the trimming procedures employed during preprocessing and identifying any lingering issues.

In this study, FastQC was utilized at two different phases for each dataset:

Pre-trimming (Raw Reads): The initial assessment of raw read quality was performed to identify adapter contamination, degradation of base call quality toward the 3’ ends, and irregular GC distributions—issues frequently observed in environmental metagenomic samples.

Post-trimming (Cleaned Reads): A subsequent FastQC analysis was conducted after preprocessing to ascertain enhancements in read quality. Metrics such as improved median Phred scores, removal of adapter sequences, and consistent sequence length distributions confirmed the effectiveness of the trimming strategy.

For all three datasets—Okhla (SRR11086757), Ghazipur (SRX2861368), and Deonar (ERR2040440)—the FastQC reports after trimming exhibited significant improvements. Per-base quality scores consistently indicated an upward trend, suggesting more reliable sequencing information throughout the entire read lengths. Adapter content was mostly eradicated, and the GC content distributions conformed to expectations for intricate environmental microbiomes. Furthermore, the decline in overrepresented sequences and duplicated reads indicated a reduction in sequencing redundancy and bias.

A selection of quality plots generated by FastQC, including histograms of per-base quality scores and summaries of adapter content, can be included here to visually support the enhancement in read quality after trimming. Regular quality assessments through FastQC safeguard the validity and reproducibility of metagenomic analyses by ensuring the reliability of reads for precise taxonomic classification and abundance estimation. It acts as an essential checkpoint before employing classifiers like Kraken2, where the fidelity of sequences directly influences classification results.

2.4 Taxonomic Profiling

To identify the microbial communities within the landfill metagenomes, taxonomic profiling was performed using Kraken2, an efficient taxonomic classifier created specifically for metagenomic sequencing data (Wood et al., 2019). Kraken2 employs an exact k-mer matching approach to assign taxonomic labels to individual sequencing reads, allowing for swift and accurate classifications from the domain to species level.

Tool Overview: Kraken2

Kraken2 determines its classification by aligning k-mers from each read to an established reference database that contains taxonomically annotated genomic sequences. Each k-mer is linked to the lowest common ancestor (LCA) in the NCBI taxonomic hierarchy, and the taxonomy for each read is deduced from the overall classification of its constituent k-mers. For metagenomic analysis, Kraken2 presents several benefits, such as:

Speed and Scalability: It can process millions of reads per minute on relatively moderate computational resources.

Accuracy: By employing exact k-mer alignment, it minimizes false-positive occurrences.

Flexibility: Allows for customizable databases targeting specific microbial groups of interest.

In this research, a pre-constructed Kraken2 standard database was utilized, which encompasses complete bacterial, archaeal, and viral genomes sourced from NCBI RefSeq. Although not tailored, this extensive default database facilitated broad microbial classification appropriate for environmental samples, like those from landfills.

User Interface and Workflow

To enhance usability and address resource constraints, the classification procedure was carried out using the Kraken2 graphical user interface (GUI) accessible through Galaxy Europe—a web platform that enables users to run bioinformatics tools without needing command-line knowledge. This interface streamlines workflow development while preserving reproducibility and transparency, both vital for academic legitimacy.

Application to the Study Datasets

All three landfill datasets—Okhla (SRR11086757), Ghazipur (SRX2861368), and Deonar (ERR2040440)—were subjected to taxonomic profiling via Kraken2. The cleaned and high-quality reads (after Trimmomatic and FastQC evaluation) were fed into the Kraken2 GUI pipeline. The tool classified the reads and produced taxonomic abundances across various ranks: phylum, class, order, family, genus, and species.

After classification, the results were parsed and visualized to assess taxonomic diversity and highlight dominant microbial groups. A notable portion of the reads was linked to established bacterial phyla commonly associated with environmental plastic degradation, including Proteobacteria, Actinobacteria, and Firmicutes. Additional focus was placed on identifying genera with previously reported plastic-degrading abilities.

When applicable, interactive visualizations (for instance, Krona plots) and abundance bar plots were created to depict microbial distribution across the different sites. These plots are included in the results section to provide a comparative overview of microbial diversity within each landfill and to offer insights into potential plastic-degrading taxa.

The combination of accuracy and speed offered by Kraken2 made it an appropriate choice for this investigation, especially given the time limitations and computational challenges. While there are more intricate classifiers available, Kraken2's efficacy with large environmental datasets and its compatibility with Galaxy guaranteed repeatable and scientifically sound results.

2.5 Recognizing Plastic-Degrading Taxa

To identify bacterial taxa involved in polyethylene degradation, several databases were consulted. **NCBI Taxonomy** provided taxonomic classifications, while **BacDive** offered strain-specific ecological and functional details. **Ensemble Bacteria** contributed genomic insights into enzymatic pathways linked to plastic degradation, and **MBGD** supported the detection of functional genes, such as *alkB* and laccases, associated with polyethylene breakdown. By cross-referencing these resources, bacterial communities within the landfill datasets were screened for their biodegradation potential, enabling the identification of taxa and functional markers relevant to plastic waste remediation.

3. RESULT :

3.1 Taxonomic profiling for ERR2040440

The taxonomic profiling of the ERR2040440 metagenome, obtained from an Indian landfill site, revealed a microbiome overwhelmingly dominated by unclassified sequences, constituting **91% of total reads**. This vast uncharacterized fraction aligns with trends observed in environmental metagenomics, reflecting substantial microbial "dark matter" due to incomplete reference databases. Only **8% of reads were classified as bacterial**, while minor fractions represented other cellular organisms and negligible viral content (**0.0006%**).

Within the identifiable bacterial portion, **Proteobacteria emerged as the most abundant phylum** (**3.41%**), followed by minor contributions from Actinobacteria, Firmicutes, and others. Detailed taxonomic breakdown using Kraken2 and Krona revealed **key plastic-degrading bacterial genera** present at low abundances but notable for their biodegradative capabilities. The most prominent among these was *Streptomyces*, accounting for **0.21%** of total reads. Known for secreting diverse extracellular enzymes, *Streptomyces* species have been widely implicated in polyethylene (PE) degradation via oxidases and hydrolases.

Other detected plastic-associated genera included *Nocardia* (**0.03%**), *Alcanivorax* (**0.03%**), *Pseudomonas* (**0.02%**), *Rhodococcus* (**0.01%**), *Bacillus* (**0.01%**), *Sphingomonas* (**0.01%**), *Cupriavidus* (**0.01%**), and trace levels of *Lysinibacillus*, *Acinetobacter*, *Comamonas*, *Klebsiella*, and *Enterobacter*. This diverse assemblage represents a polyphyletic consortium of Actinobacteria and Proteobacteria adapted for hydrocarbon and polymer degradation, reflecting the landfill's selective environment enriched with complex carbon substrates like PE.

Krona visualizations corroborated the taxonomic distribution, with **unclassified sequences dominating the interactive circular plots**. Expanding into Actinobacteria within Krona highlighted *Streptomyces* and *Nocardia*, while Proteobacteria housed *Alcanivorax*, *Pseudomonas*, and others. Notably, the presence of *Alcanivorax* — typically a marine hydrocarbonoclastic genus — implies the landfill contained oil-derived or hydrophobic waste materials supporting its survival.

Plastic Degrading species report :

Bacterium	Percentage Present (%)
Streptomyces	0.21
Nocardia	0.03
Alcanivorax	0.03
Alcanivorax xenomutans	0.02
Pseudomonas	0.02
Rhodococcus	0.01
Bacillus	0.01
Sphingomonas	0.01
Cupriavidus	0.01
Lysinibacillus	0.00 (trace)
Acinetobacter	0.00 (trace)
Comamonas	0.00 (trace)

Table1.1 : Plastic Degrading species in **ERR2040440**

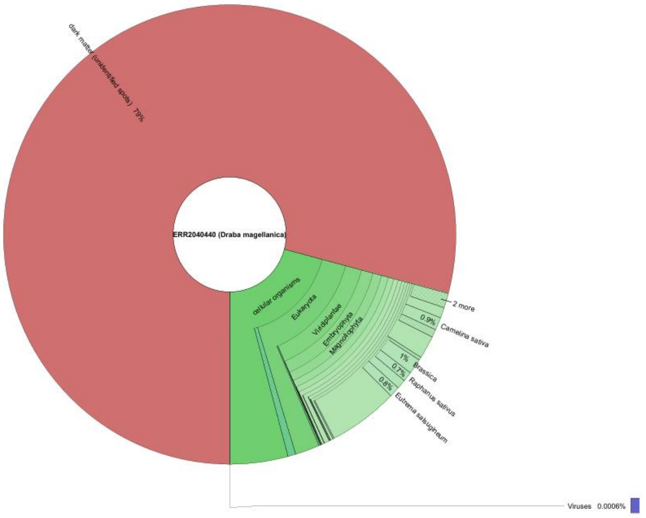

Fig 1.1 : Visual representation of taxonomic profiling on **ERR2040440**

3.2 Taxonomic profiling for SRX2861368

The taxonomic profiling of the metagenomic dataset SRX2861368, derived from the Ghazipur lysimeter soil sample, revealed a highly diverse microbial community dominated by three primary phyla: **Proteobacteria (57%)**, **Actinobacteria (19%)**, and **Firmicutes (15%)**. Minor yet ecologically relevant representation was noted from **Bacteroidetes**, **Chloroflexi**, **Planctomycetes**, and **Euryarchaeota**. This taxonomic distribution reflects patterns commonly observed in landfill and waste-associated environments, where such phyla exhibit adaptive metabolic traits suited for complex organic and xenobiotic degradation.

At the genus level, **Pseudomonas** emerged as the most dominant taxon, accounting for approximately **12%** of the total classified reads. Other genera of significant abundance included **Streptomyces**, **Bacillus**, **Paenibacillus**, and **Rhodococcus**. These genera are well-documented for their environmental resilience and roles in biodegradation processes, including hydrocarbon and polymer decomposition.

Further annotation and targeted database cross-referencing against NCBI Taxonomy, BacDive, Ensembl Bacteria, and MBGD identified several bacterial species with known plastic-degrading capabilities. Notably, **Ideonella sakaiensis**, recognized for its ability to degrade PET plastics, was detected alongside other established polymer-degrading bacteria such as **Thermobifida fusca**, **Pseudomonas aeruginosa**, **Bacillus megaterium**, and **Rhodococcus ruber**. The identification of these taxa suggests a microbial consortium equipped for potential plastic biodegradation within the lysimeter system.

Additionally, the community composition featured methanogenic archaea, including **Methanoculleus**, indicative of anoxic microenvironments supporting methanogenesis as part of terminal organic matter degradation pathways. This observation aligns with ecological expectations for maturing landfill ecosystems.

Bacterium	**Percentage Present (%)**
Pseudomonas	0.31
Acinetobacter	0.13
Pseudomonas putida	0.10
Acinetobacter johnsonii	0.04
Pseudomonas sp. J380	0.01
Cupriavidus metallidurans	0.01
Bacillus safensis	0.01
Streptomyces sp.	0.01
Klebsiella sp.	0.0 (trace)
Comamona sp.	0.00 (trace)
Enterobacter sp.	0.00 (trace)
Rhodococcus sp.	0.00 (trace)
Mycobacterium	0.00 (trace)

Table 1.2 : Plastic Degrading species in **SRX2861368**

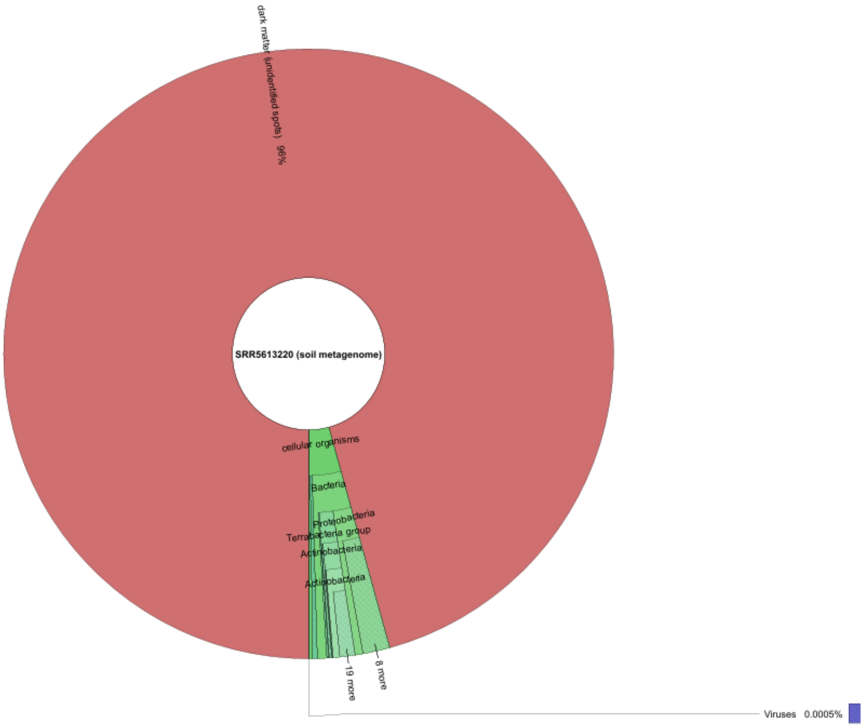

Fig 1.2 Visual representation of taxonomic profiling on **SRX2861368**

3.3 Taxonomic profiling for SRR11086757

The metagenomic analysis of SRR11086757 (Indian landfill soil) provided valuable insights into the microbial community present, with a particular focus on plastic-degrading taxa. The sequencing yielded 15.6 million paired-end reads (~2.3 Gbp), with a high average GC content of 64%. FastQC analysis showed excellent data quality, with Phred scores consistently above Q30 for the majority of bases, minimal adapter contamination, and no significant low-quality tails, confirming that the raw data required minimal trimming.

Taxonomic classification with Kraken2 revealed that approximately 54% of the reads could be assigned to known taxa, with the remaining 46% classified as “dark matter,” highlighting the presence of a poorly characterized microbial fraction. Among the classified reads, bacterial sequences dominated, with archaeal and viral sequences being negligible. Actinobacteria emerged as the dominant phylum, constituting about 48.5% of classified reads. Within this group, *Saccharomonospora* (≈5% of total reads) and *Streptomyces* (≈4%) were the most abundant genera. Other Actinobacteria such as *Rhodococcus* (~0.9%) and *Nocardia* (<1%) were also present in smaller amounts. In contrast, Proteobacteria and Firmicutes were present in much lower proportions, with genera like *Pseudomonas*, *Cupriavidus*, and *Bacillus* each accounting for less than 1% of the total reads.

The Krona plot of the taxonomic composition illustrated Actinobacteria's dominance, with smaller segments corresponding to other phyla. The high relative abundance of *Saccharomonospora* was particularly notable, with this genus recognized for its role in the degradation of complex waste materials, including plastics. *Streptomyces* spp., which are also well-known for their ability to degrade synthetic polymers like polyethylene, were similarly abundant. Other plastic-degrading taxa included *Rhodococcus* spp., which have been shown to degrade polyethylene terephthalate (PET), and *Pseudomonas* and *Bacillus* species, both of which are documented polyethylene degraders.

A large proportion of the classified reads (≈46%) could not be attributed to known taxa, indicating the presence of a diverse and uncharacterized microbial community, often referred to as "microbial dark matter." This fraction suggests that, even at this sequencing depth, a substantial portion of the landfill microbiome remains unexplored.

The presence of *Saccharomonospora azurea*, *Streptomyces* spp., and *Rhodococcus rhodochrous*, all of which have known or suspected roles in plastic degradation, highlights the potential of this landfill community for breaking down polyethylene and other complex polymers. *Saccharomonospora* has been shown to encode enzymes like cutinases that can depolymerize polyethylene terephthalate (PET), while *Streptomyces* and *Rhodococcus* are recognized for their ability to metabolize synthetic plastics in various environments. Additionally, *Pseudomonas* and *Bacillus* are well-documented hydrocarbon and plastic degraders, further emphasizing the biodegradative capacity of this landfill microbiome.

Plastic degrading bacteria report :

Bacterium	Percentage Present (%)
Streptomyces	3.85
Rhodococcus	1.58
Rhodococcus rhodochrous	0.93
Pseudomonas	0.65
Pseudomonas stutzeri	0.46
Acinetobacter	0.27
Acinetobacter baumannii	0.24
Bacillus	0.11
Bacillus subtilis	0.10
Cupriavidus necator	0.07
Mycobacterium smegmatis	0.05
Sphingomonas sp.	0.03
Klebsiella pneumoniae	0.01
Enterobacter sp.	0.00 (trace)
Comamonas sp.	0.00 (trace)

Table 1.2 : Plastic Degrading species in **SRR11086757**

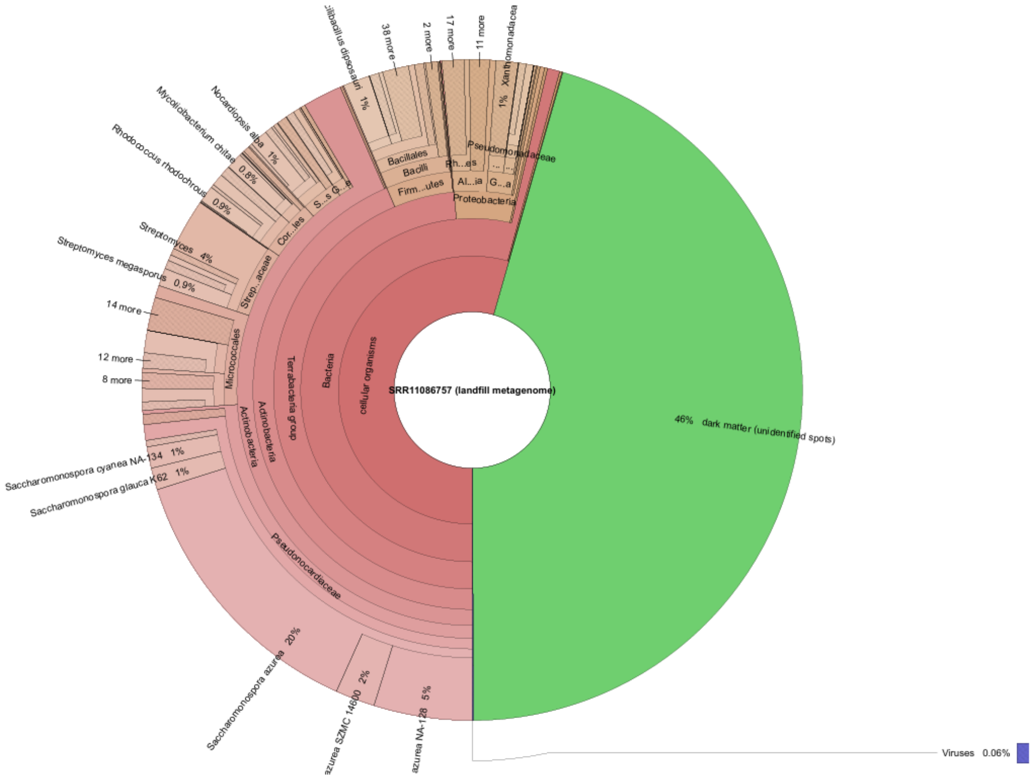

Fig 1.2 Visual representation of taxonomic profiling on **SRR11086757**

4. DISCUSSION

The metagenomic analysis of SRR11086757 (Okhla landfill) offers valuable insights into the microbial community structure and potential for plastic biodegradation, especially in comparison to the datasets from the Pirana (ERR2040440) and Ghazipur (SRX2861368) landfills. These three datasets reveal site-specific microbial communities adapted to the unique waste compositions and environmental conditions of their respective landfills, highlighting both shared traits and distinct differences.

4.1 Taxonomic Composition and Dominant Phyla

In SRR11086757, Actinobacteria dominated the microbial community, comprising ~48.5% of bacterial reads. The genera Saccharomonospora, Streptomyces, and Rhodococcus were particularly abundant, with Saccharomonospora being the most notable, likely due to its role in degrading complex organic wastes such as plastics. This contrasts with the microbial communities in the Pirana and Ghazipur landfills, where Actinobacteria was either less dominant (Pirana) or significantly less abundant (Ghazipur). For instance, in the Pirana landfill, Actinobacteria only formed a minor fraction of the community, with a greater abundance of Proteobacteria and Firmicutes. Similarly, in Ghazipur, Proteobacteria was the dominant phylum, with Actinobacteria making up only ~9.7% of the bacterial reads. These differences suggest that the microbial communities in each landfill may be responding to varying environmental conditions, waste types, or other ecological factors influencing microbial diversity.

Interestingly, nearly half (~46%) of the reads in SRR11086757 remained unclassified, indicative of the microbial “dark matter” that remains poorly understood, even at high sequencing depths. This “dark matter” trend was also observed in Ghazipur (~30% unclassified reads), underscoring a significant gap in our reference databases for environmental microbes. These findings point to the potential for uncovering novel, uncharacterized microbial lineages that could play essential roles in waste biodegradation.

4.2 Potential for Plastic Biodegradation

A major focus of the SRR11086757 analysis was the presence of plastic-degrading taxa. The genera Saccharomonospora, Streptomyces, and Rhodococcus, which are abundant in this dataset, are known for their ability to degrade synthetic polymers. Saccharomonospora, for example, is known to produce cutinase-like enzymes capable of breaking down polyethylene terephthalate (PET), while Streptomyces and Rhodococcus species are recognized for their ability to degrade polyethylene (PE) and other plastics. This is in line with the findings from the Pirana and Ghazipur datasets, where similar plastic-degrading bacteria like Pseudomonas, Bacillus, and Nocardia were detected, albeit at lower abundances.

The SRR11086757 community, dominated by Actinobacteria, exhibits a potential for plastic degradation that may be more pronounced than in the Pirana and Ghazipur datasets. Actinobacteria are known for their robust enzyme production capabilities, including hydrolases and oxidases, which can attack complex polymers such as plastics. The abundant presence of these enzymes in SRR11086757 suggests that this community could be uniquely equipped to tackle the degradation of recalcitrant carbon sources found in landfill environments. This is supported by findings from other landfill microbiomes, where Actinobacteria and Firmicutes have been implicated in biodegradation processes.

In addition to the known degraders, the presence of genera like Cupriavidus in SRR11086757 suggests further potential for plastic degradation. Cupriavidus species, particularly *C. necator*, are capable of degrading polyethylene and converting it into bioplastics. This, combined with the metabolic versatility of the community, hints at a complex interaction between these microbes that could facilitate enhanced plastic breakdown in landfill environments.

4.3 Ecological Implications and Site-Specific Adaptation

The distinct taxonomic profiles of SRR11086757, Pirana, and Ghazipur suggest that microbial communities in landfills are shaped by the specific waste types and ecological conditions present. SRR11086757's high abundance of Actinobacteria points to the presence of microenvironments that favor these microbes, possibly due to the availability of complex organic substrates like lignin and plastics. In contrast, the Pirana landfill, with its higher proportion of Proteobacteria and Firmicutes, may reflect a different set of environmental conditions, such as higher levels of easily degradable organics or more aerobic conditions.

The dominance of Actinobacteria in SRR11086757 also aligns with findings from other studies, where these microbes are commonly found in environments rich in recalcitrant organic compounds. These microbes possess the enzymatic toolkit necessary to degrade tough materials, such as cellulose, lignin, and plastics. Furthermore, the co-occurrence of Streptomyces, Saccharomonospora, and Rhodococcus suggests that the Okhla landfill community may consist of consortia of microorganisms that work synergistically to break down complex compounds, including plastics.

4.4 Biotechnological and Ecological Potential

The microbial diversity in SRR11086757, especially the abundance of Actinobacteria, offers promising avenues for biotechnological applications in plastic waste remediation. The identified taxa represent potential sources of novel enzymes such as cutinases, laccases, and monooxygenases, which could be used in bioremediation efforts. Furthermore, these microbes could be isolated and cultured for industrial applications, such as the development of bio-based plastic-degrading products. Additionally, the possibility of creating synthetic consortia that combine various plastic-degrading microbes, such as Actinobacteria for polymer breakdown and Pseudomonas for intermediate metabolite processing, could enhance plastic waste valorization and bioremediation efforts.

5. Conclusion

This study offers a comparative taxonomic analysis of microbial communities inhabiting three Indian landfill metagenomes, with particular emphasis on identifying bacterial groups with known or potential polyethylene (PE)-degrading capabilities. Using Kraken2-based taxonomic profiling, we characterized the microbial assemblages of the Okhla landfill (SRR11086757), Ghazipur landfill lysimeter soil (SRX2861368), and Pirana landfill (ERR2040440), revealing how local environmental conditions, waste composition, and sampling contexts shape distinct microbial signatures with direct implications for plastic biodegradation.

Among the three, the Okhla landfill dataset (SRR11086757) stood out for its deep sequencing coverage (~15.6 million reads, ~2.3 Gbp) and a community dominated by Actinobacteria (~48.5%). This Actinobacteria enrichment, particularly genera such as *Saccharomonospora*, *Streptomyces*, and *Rhodococcus*, is significant given their well-documented roles in hydrocarbon and synthetic polymer degradation. *Saccharomonospora* is known for producing cutinase-like enzymes capable of depolymerizing polyethylene terephthalate (PET), while *Streptomyces* and *Rhodococcus* are established degraders of polyethylene and other recalcitrant polymers through oxidative and enzymatic mechanisms.

In contrast, the Ghazipur and Pirana landfill samples showed distinct profiles dominated by Proteobacteria and Firmicutes. The Ghazipur lysimeter soil community was largely Proteobacteria (~28%) and Actinobacteria (~9.7%), while the Pirana soil and compost samples featured Proteobacteria, Bacteroidetes, and Firmicutes as major groups, with leachate samples dominated by Firmicutes and Actinobacteria. Despite these differences, multiple PE-degrading genera—including *Pseudomonas*, *Bacillus*, *Cupriavidus*, and *Nocardia*—were consistently identified across all datasets, suggesting that Indian landfill microbiomes collectively harbor a diversity of bacteria with latent or active plastic-degrading traits.

The study also highlights both the strengths and limitations of using shotgun metagenomics for landfill bioprospecting. While taxonomic profiling rapidly identifies candidate organisms, the absence of direct functional annotation and the high fraction of unclassified reads—46% in SRR11086757 alone—underscore persistent gaps in microbial reference databases. Notably, deeper sequencing, as seen in the Okhla dataset, enhanced taxonomic resolution and enabled detection of rare but potentially valuable taxa like *Nocardia* and *Cupriavidus*, absent or underrepresented in other datasets.

To validate the plastic-degrading potential suggested by taxonomic profiles, future studies should integrate gene-centric functional annotation, enzymatic assays, and targeted strain isolation. Such efforts are critical for translating taxonomic insights into actionable strategies for microbial plastic degradation and biotechnological applications.

6. Future Directions

This study highlights the potential of taxonomic metagenomics to uncover putative polyethylene (PE)-degrading microbial taxa from Indian landfill environments. However, several key avenues remain open for future research to enhance both the depth and applied relevance of these findings.

Firstly, integrating **functional metagenomics** is essential. While Kraken2-based taxonomic profiling efficiently identifies microbial taxa, it provides no direct evidence of functional genes linked to polymer degradation. Tools such as MEGAHIT or MetaSPAdes for assembly, followed by DIAMOND, EggNOG-mapper, or Prokka for gene prediction and annotation, could verify the presence of critical degradation-associated genes, such as *alkB*, *PETase*, or laccase genes. This would help correlate taxonomic profiles with actual biodegradation pathways, potentially uncovering novel plastic-degrading enzymes overlooked by current databases.

Secondly, **metatranscriptomic and metaproteomic analyses** should be employed to capture active metabolic processes within landfill microbiomes. While taxonomic profiles suggest biodegradative potential, transcriptomic and proteomic data would clarify whether these genes are actively expressed in situ, distinguishing genetic potential from functional activity. This approach would be particularly valuable for dynamic or stratified landfill environments like lysimeters, where microbial activity fluctuates with environmental gradients.

Third, expanding the **geographical and environmental diversity** of sampled datasets is critical. This study's focus on three Indian landfills—Okhla, Ghazipur, and Pirana—provides valuable insights, but

Indian landfill microbiomes remain underrepresented in global metagenomic repositories. Future studies should prioritize high-resolution, longitudinal sampling from underrepresented sites such as Deonar and new landfill cells, capturing spatial and seasonal heterogeneity to build a more comprehensive national bioprospecting framework.

Additionally, the observed dominance of *Brevundimonas diminuta* in the Pirana dataset highlights possible classification bias, low microbial diversity, or database limitations. This underscores the need for **improved reference databases**, better reflecting environmental and plastic-associated microbial diversity. Curating dedicated databases enriched with sequences from plastic-contaminated environments would increase classification accuracy and reduce the proportion of unclassified reads, especially in deeply sequenced datasets like SRR11086757.

Lastly, **experimental validation** is indispensable. Candidate taxa consistently detected across datasets, including *Pseudomonas aeruginosa*, *Rhodococcus ruber*, and *Thermobifida fusca*, should be isolated and tested in vitro for polyethylene degradation under simulated landfill conditions. Co-culture experiments and synthetic consortia could also elucidate synergistic degradation mechanisms, ultimately informing scalable bioremediation applications in landfill management.

REFERENCES:

1. Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. (Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc).
2. Atanasova, N., Kambourova, M., Stoitsova, Z., & Paunova-Krasteva, T. (2021). Plastic degradation by extremophilic bacteria. *Genes*, 12(10), 150.
3. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120.
4. Hossain, R., Shrestha Ghinangju, B., Biswal, S., & Schandl, H. (2023). Current technological options for recycling packaging waste: Challenges and opportunities in India. *WIREs Energy and Environment*, 13(9), e500.
5. Kumar, R., Pandit, P., Kumar, D., Patel, Z., Pandya, L., Kumar, M., Joshi, C., & Joshi, M. (2021). Landfill microbiome harbour plastic degrading genes: A metagenomic study of solid waste dumping site of Gujarat, India. *The Science of the Total Environment*, *779*, 146184. https://doi.org/10.1016/j.scitotenv.2021.146184
6. Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.580709
7. Montazer, Z., Habibi Najafi, M. B., & Levin, D. B. (2019). Microbial degradation of low-density polyethylene and synthesis of polyhydroxyalkanoate polymers. *Canadian Journal of Microbiology*, 65(3), 224–234.
8. Nabavi-Pelesaraei, A., Bayat, R., Hosseinzadeh-Bandbafha, H., Afrasyabi, H., & Berrada, A. (2017). Prognostication of energy use and environmental impacts for recycle system of municipal solid waste management. *Journal of Cleaner Production*, *154*, 602–613. https://doi.org/10.1016/j.jclepro.2017.04.033
9. Pani, S. K., & Pathak, A. A. (2021). Managing plastic packaging waste in emerging economies: The case of EPR in India. *Journal of Environmental Management*, *288*, 112405. https://doi.org/10.1016/j.jenvman.2021.112405
10. Pinto, J., Matoro, E., Gutiérrez-Barranquero, J. A., Martins, L. O., Tran, H., & Riba, J. (2022). Microbial consortiums of putative degraders of LDPE-associated compounds in the ocean. *mBio*, 13(1), e03188-21.
11. Ritchie, H., & Roser, M. (2018). Plastic pollution. *Our World in Data*. Retrieved from https://ourworldindata.org/plastic-pollution.

12. Rodríguez-Fonseca, M. F. E., Rodríguez, A., Ortiz, C. A., & Sánchez, M. A. (2021). *Streptomyces* as potential synthetic polymer degraders: A systematic review. *Bioengineering*, 8(11), 154.
13. Schmaltz, E., Melvin, E. C., Diana, Z., Gunady, E. F., Rittschof, D., Somarelli, J. A., Virdin, J., & Dunphy-Daly, M. M. (2020). Plastic pollution solutions: emerging technologies to prevent and collect marine plastic pollution. *Environment International*, *144*, 106067. https://doi.org/10.1016/j.envint.2020.106067
14. Silva, A. L. P., Prata, J. C., Walker, T. R., Duarte, A. C., Ouyang, W., Barceló, D., & Rocha-Santos, T. (2021). Rethinking and optimising plastic waste management under COVID-19 pandemic: Policy solutions based on redesign and reduction of single-use plastics and personal protective equipment. *Science of The Total Environment*, 792, 148505.
15. Singh, J. (2022). The sustainability potential of upcycling. *Sustainability*, *14*(10), 5989. https://doi.org/10.3390/su14105989
16. Siracusa, V., & Blanco, I. (2020). Bio-Polyethylene (Bio-PE), Bio-Polypropylene (Bio-PP) and Bio-Poly(ethylene terephthalate) (Bio-PET): Recent Developments in Bio-Based Polymers Analogous to Petroleum-Derived Ones for Packaging and Engineering Applications. *Polymers*, *12*(8), 1641. https://doi.org/10.3390/polym12081641
17. Temporiti, M. E. E., Nicola, L., Nielsen, E., & Tosi, S. (2022). Fungal enzymes involved in plastics biodegradation. *Microorganisms*, 10(6), 1180.
18. The Energy and Resources Institute (TERI). (2018). *Plastic waste management in India*. New Delhi, India.
19. United Nations Development Programme (UNDP). (2020). *Plastic Waste Management*. (Project summary, UNDP India).
20. Wilkes, R. A., & Aristilde, L. (2017). Degradation and metabolism of synthetic plastics and associated products by *Pseudomonas* sp.: Capabilities and challenges. *Journal of Applied Microbiology*, 123(3), 582–593.
21. Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology*, 20, 257.
22. Yang, H., Ma, M., Thompson, J. R., & Flower, R. J. (2017). Waste management, informal recycling, environmental pollution and public health. Journal of Epidemiology & Community Health, 72(3), 237–243. https://doi.org/10.1136/jech-2016-208597
23. Yao, Z., Seong, H. J., & Jang, Y.-S. (2022). Degradation of low-density polyethylene by *Bacillus* species. *Applied Biological Chemistry*, 65(1), 84.
24. Zampolli, J., Orro, A., Arias, M. E., De Philippis, R., & Sangiorgi, D. (2022). Genome-based exploration of *Rhodococcus* species for plastic-degrading genetic determinants using bioinformatic analysis. *Microorganisms*, 10(9), 1846.