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Isolation and Characterization of Polyethylene-Degrading Bacteria from Municipal Dumpsite Soils of Gujarat, India

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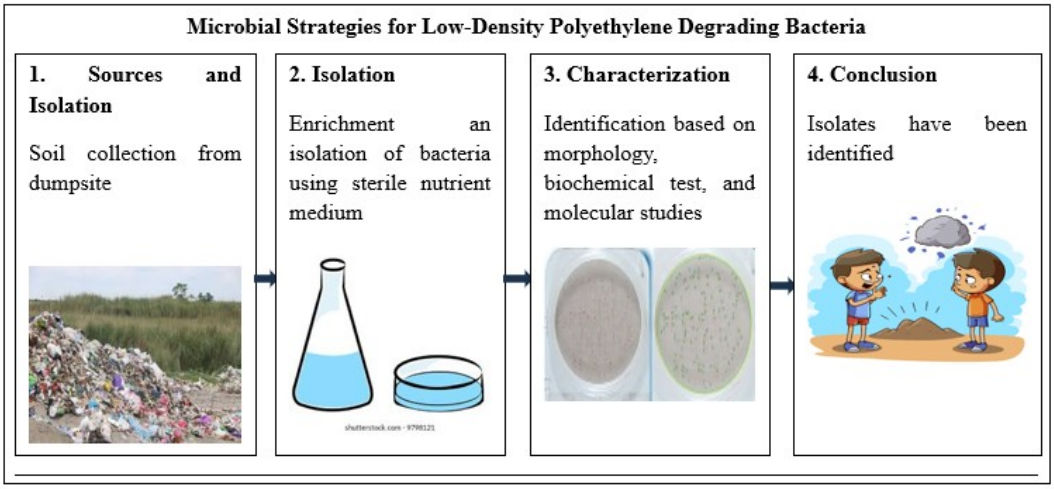
ABSTRACT:

Plastic waste accumulation, particularly polyethylene, poses a significant environmental challenge due to its recalcitrant nature and persistence in ecosystems. The present study aimed to isolate, characterize, and evaluate polyethylene-degrading bacteria from municipal dumpsite soils collected from Kalol (Gandhinagar) and Pirana (Ahmedabad), Gujarat, India. Soil samples were enriched in mineral salt medium (MSM) supplemented with polyethylene as the sole carbon source, followed by isolation and characterization of bacterial strains using morphological, biochemical, and molecular approaches. Two bacterial isolates were obtained and identified through Gram staining, biochemical profiling, and 16s rRNA gene sequencing as Bacillus paramycoides and Pseudomonas aeruginosa. Biochemical analysis revealed distinct metabolic capabilities, with Pseudomonas aeruginosa exhibiting stronger metabolic activities associated with polymer degradation. In weight-loss assays, Bacillus paramycoides and Pseudomonas aeruginosa caused 3.59 ± 0.01 % and 4.85 ± 0.24 % mass loss of polyethylene films, respectively, after 90 days of incubation in MSM. FTIR analysis confirmed the appearance of new oxygen-containing functional groups on the polymer surface after bacterial treatment, indicating oxidative degradation of polyethylene. These findings demonstrate that municipal dumpsite soils are valuable reservoirs of polyethylene-degrading bacteria and highlight the potential application of Bacillus paramycoides and Pseudomonas aeruginosa in eco-friendly plastic waste management strategies.

Keywords: Isolation, Characterization, LDPE degrading bacteria, 16s rRNA sequencing, weight loss, FTIR



1 INTRODUCTION:

Polyethylene is the most widely used plastic globally due to its low cost, low density, and mechanical flexibility; however, its high durability leads to persistent accumulation of polyethylene waste in the

environment [1]. Bioremediation is a low-cost, environmentally friendly approach that uses microorganisms to remove or detoxify a wide range of environmental pollutants. While, traditional approaches, the use of microorganisms can effectively degrade polymers, polyaromatic hydrocarbons, phenols, pesticides [2].

Biosurfactant compounds are formed of molecules that have hydrophobic and hydrophilic ends, which include acids, ionized peptides, mono, di, or polysaccharides, and a hydrophobic moiety composed of fatty acids or saturated/un-saturated hydrocarbon chains due to the amphiphilic property that allows for the extension of the surface of the hydrophobic substances, which are insoluble in water, increase the bioavailability of these substances in water, and change the properties of bacterial cells [3]. The research found that the amount of CI extracted from samples collected by PP after 10 years was double of what was obtained after 10 years and from fresh PP [4]. The study has identified an innovative enzyme, which degrades PE, known as PEase, for polyethylene biodegradation. It also enhances the knowledge regarding the LDPE degrading bacteria, named *Rhodococcus sp. C-2*, obtained from seawater [5].

Some of the studied bacteria were found to be *Pseudomonas sp.*, *Streptococcus sp.*, *Staphylococcus sp.*, *Micrococcus sp.*, *and Moraxella sp.* The bacteria used were also *Bacillus subtilis*, *Bacillus amylolyticus*, and *Arthrobacter defluvii*; the results showed that the degradation ability of *Bacillus subtilis* was lower than other organisms [6]. In the study, it is indicated that *Bacillus sp.* have been cultivated on a medium with LDPE for observing their colonization on a hydrophobic surface and there has also been shown a reduction in the weight of LDPE by 1.5 % through the process of colonization using *Bacillus sp. (ISJ55)* after 60 days of incubation [7].

The research suggests that the utilization of different strategies such as improving microbial cell-surface hydrophobicity or oxidation of LDPE prior to biodegradation by *Enterobacter cloacae AKS7* can be used for degradation of LDPE molecules [8].

2 MATERIAL AND METHOD:

2.1 Sample collection:

Soil samples were collected from municipal dumpsites at Kalol (Gandhinagar) and Pirana (Ahmedabad), Gujarat, using clean, sterilized containers. From each site, composite soil samples were taken from a depth of 15 cm below the surface [9]. Samples were transported to the laboratory and stored at 4 ℃ until the experiments were carried out. The first dump site is located at Kalol, Gandhinagar, Gujarat, India, (23° 15' 0" North, 72° 29' 0" East), and second dumpsite is located at Pirana, Ahmedabad, Gujarat, India, (22° 98' 0" North, 72° 56' 0" East) as shown in fig 1 and 2.

Fig 1. Soil sample collection from dumpsite location (a) is situated in Kalol, Gandhinagar, Gujarat, India, (23° 15' 0" North, 72° 29' 0" East) (b) is situated in Pirana, Ahmedabad, Gujarat, India, (22° 98' 0" North, 72° 56' 0" East).

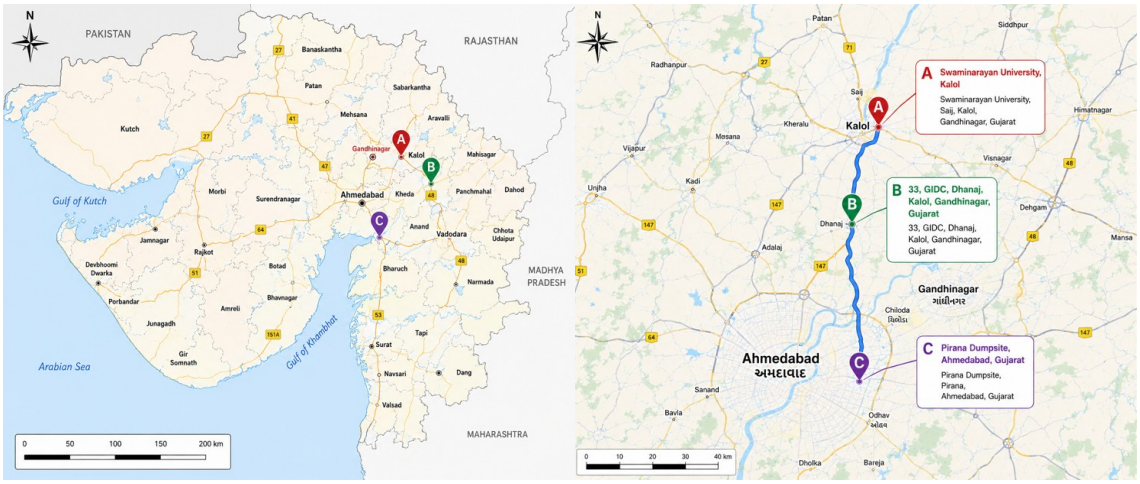


Fig 2. Locations of (a) Gujarat map (b) Locations map of soil sample collection: A. Swaminarayan University, Kalol, Gandhinagar, Gujarat; B. GIDC, Dhanaj, Kalol dumpsite, Gandhinagar, Gujarat; C. Pirana dumpsite, Ahmedabad, Gujarat.

2.2 Enrichment and isolation medium:

For enrichment of LDPE-degrading bacteria, soil suspensions were inoculated into sterile nutrient broth containing mineral salt medium (MSM) and incubated at 37 ℃ for 24 hours. The components of the medium were as follows: KH_2PO_4: 1.5 g/L, Na_2HPO_4: 1.5 g/L, $MgSO_4 \cdot 7H_2O$: 0.2 g/L, NH_4NO_3: 1.0 g/L, $FeSO_4 \cdot 7H_2O$: 0.05 g/L, $CaCl_2$: 0.02 g/L, Trace elements: $MnSO_4$: 0.01 g/L, $ZnSO_4$: 0.01 g/L, $CuSO_4$: 0.01 g/L, $CoSO_4$: 0.01 g/L, Na_2MoO_4: 0.01 g/L [10]. The enriched culture was then streaked on nutrient agar medium and sub cultured to get pure colonies. The plates were incubated at 37℃ for 24 hours, after which the isolates were characterized based on morphology, colony appearance, and biochemical tests [11]. Isolated colonies were stored at 4 ℃.

2.3 Identification of isolated bacteria:

Bacteria identification was carried out through Gram staining, biochemical tests, and 16s rRNA gene sequencing. Colony morphology, which includes colony size, shape, elevation, surface type, margin, transparency, color, odor, and pigmentation was observed in nutrient agar media. Cellular morphology was determined through Gram staining, whereas biochemical properties were assessed through biochemical tests [12]. Biochemical profiles and Gram-stain characteristics were interpreted with reference to Bergey's Manual of Systematic Bacteriology [13,14].

2.4 16s rRNA genome sequencing:

Samples from various strains generated from PCR in the experiment were sent to Gujarat Biotechnology Research Centre, Gandhinagar, Gujarat, for the sequencing of 16S rRNA gene. The genomic DNA was isolated using the PrepMan Ultra (Thermo fisher) reagent kit. 16s rRNA gene of bacteria was amplified using Takara EmeraldAmp® GT Master Mix using universal 16s rRNA primers 27F and 1492R and amplification reactions were conducted using the Veriti Thermal Cycler. The amplified products were purified using ExoSAP-IT™ Express which removed excess primers and nucleotides from PCR products. The purified PCR products were then used as templates for the sequencing reaction. Big dye terminator v3.1 chemistry was used to carry out reactions using primers 27F and 1492R which were purified using Ethanol precipitation method. Finally, sequencing products were sequenced using Sanger sequencing loaded into the 3500XL Genetic Analyzer. The electropherograms obtained were analyzed using SeqA6 software. The final sequence was compared with sequences available in NCBI BLASTn against 16s rRNA gene database (Bacteria). Accession numbers PZ134689 and PZ134699 were assigned for *Pseudomonas aeruginosa* and *Bacillus paramycoides*, respectively [15]. Phylogenetic analysis of the sequences was done using the MEGA12 program.

2.5 Biodegradation Assay: Weight loss study

Pure microbial isolates were inoculated into 100 mL MSM containing 5% (v/v) actively growing culture and a 2 × 2 cm polyethylene film as the sole carbon source [16]. Biodegradation assays were conducted in triplicates (n=3) by incubating the cultures at 37°C under static conditions for 90 days. Polyethylene samples were retrieved at 15 days intervals, washed, dried, and weighed to determine percentage weight loss. The percentage weight loss was calculated by following equation (1) [17].

$$Weight\ loss(\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100 \quad \textbf{(1)}$$

2.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis:

Changes in polymer bonds following biodegradation were determined using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy. After 90 days of bacterial treatment, LDPE films were harvested, washed with 2% SDS, vacuum-dried, and scanned over a wavelength range of 4000–500 cm^{-1}. Polymer degradation was assessed based on alterations or modifications in functional groups and reductions in the transmission intensity of characteristic polymer bonds.

3 RESULT AND DISCUSSION

After enrichment, the isolates were streaked on agar plates and incubated for 24 hours at 37°C, resulting in 15 distinct colonies. The colonies were then grown individually on MSM agar plates, where polyethylene was the sole source of carbon. Only two of the 15 isolates grew on these plates, suggesting their ability to degrade polyethylene.

The method of using polyethylene strips in isolating microorganisms had been extensively covered by numerous studies before. According to the results of the study, isolate SARR1 had been shown to degrade the LDPE at the rate of 0.069 g per day, and the LDPE strips have a half-life of 335.32 days to degrade LDPE strips [18]. Kyaw et al. researched the biodegradability of LDPE through four strains of *Pseudomonas sp.: Pseudomonas aeruginosa PAO1 (ATCC 15729)*, *Pseudomonas aeruginosa (ATCC 15692)*, *Pseudomonas putida (KT2440 ATCC 47054)*, and *Pseudomonas syringae (DC3000 ATCC 10862)* [19]. Hussein, *et al*. had been isolated three isolates and identified as *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Acinetobacter ursingii* [20].

Bacteria were identified and classified using the Bergey's Manual of Systematic Bacteriology. From all the isolated bacteria, only two species (IS1 and IS2) could grow on MSM containing polyethylene as the sole carbon source. Gram staining revealed that IS1 was Gram-positive while IS2 was Gram-negative.

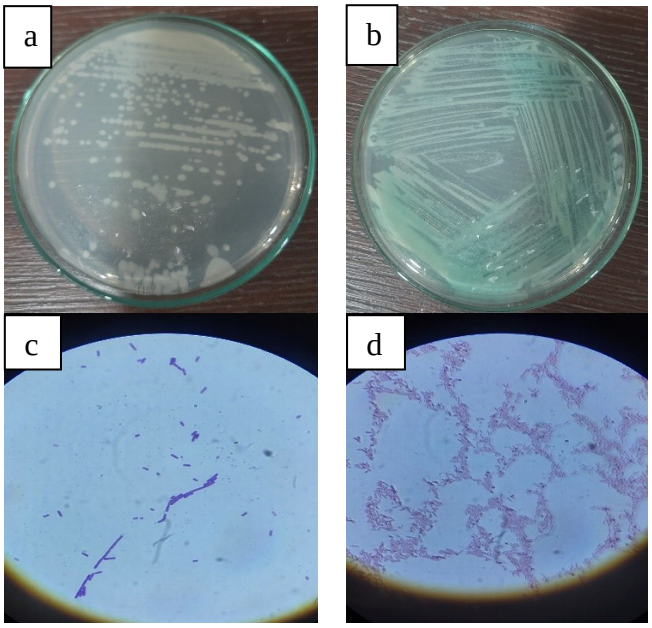


Fig. 3. Screening for polyethylene degrading bacteria. Both (a) and (b) show polyethylene degrading isolates grown on basal medium supplemented with polyethylene. Gram's staining of isolated polyethylene degrading bacteria viewed under 100x magnifications. (c) Gram- positive rod shaped and (d) Gram- negative rod shaped isolated from dumpsite.

The following were various biochemical tests used in bacterial identification: Carbohydrate Utilization Test, Citrate Utilization Test, Nitrate Reductase Test, Starch Hydrolysis Test, Vogues Proskauer's Test, Methyl Red Test, Indole Test, H_2S Production Test, and Oxidase Test, Gelatine Test, Motility Test, and Lipase Test on biochemical medium [21]. Out of which IS1 showed positive reactions for D-Glucose, Mannitol, Citrate Utilization Test, Nitrate Reductase Test, Starch Hydrolysis Test, and V-P test whereas IS2 showed positive reactions for D-Glucose, Fructose, Mannitol, Citrate Utilization Test, Nitrate Reductase Test, Oxidase Test, Catalase Test, Gelatine Test, Motility Test, and Lipase Test. Both bacteria were previously isolated with great ability to degrade LDPE [22,23,24,25,26,27,28.29].

Table 1. Identification of IS1 and IS2 for LDPE degradation

Isolates test	IS1	IS2
Morphology Gram's reaction	Gram + Ve Rod	Gram - Ve Rod
D- Glucose	+ Ve	+ Ve
Mannose	- Ve	- Ve
Fructose	- Ve	+ Ve
Mannitol	+ Ve	+ Ve
Citrate Utilization Test	+ Ve	+ Ve
Nitrate Reductase Test	+ Ve	+ Ve
Starch Hydrolyzing Test	+ Ve	- Ve
Vogues Proskauer's Test	+ Ve	- Ve
Methyl Red Test	- Ve	- Ve
Indole Test	- Ve	- Ve
H_2S Production Test	- Ve	- Ve
Oxidase Test	- Ve	+ Ve
Catalase Test	- Ve	+ Ve
Gelatine Test	- Ve	+ Ve
Motility Test Lipase Test	- Ve	+ Ve
Lipase Test	- Ve	+ Ve
Probable organism	*Bacillus sp.*	*Pseudomonas sp.*

Phylogeny of the strains was done by computational tool MEGA12 software. On the basis of analysis of 16S rRNA gene sequence, the strains IS1 and IS2 were identified to be *Bacillus paramycoides* and *Pseudomonas aeruginosa*, respectively. The strains showed the capacity to degrade low-density polyethylene (LDPE). In addition, *Alcaligenes faecalis (MK517568)* and *Bacillus cereus (MK517567)* had been characterized to be polyethylene degrading bacteria isolated from municipal dumping sites [30]. *Aspergillus flavus* and *Aspergillus terreus* were found to have the potential for the degradation of LDPE at municipal landfill sites in Agra [31]. Also, phylogenetic analysis of the three bacterial isolates revealed that the sequences of isolates *KS35* had similarity to *Methylobacterium radiotolerans MN525302*, *KS119* had similarity with *Methylobacterium fujisawaense KT720189*, and *KS14* with species *Lysinibacillus fusiformis* [32].

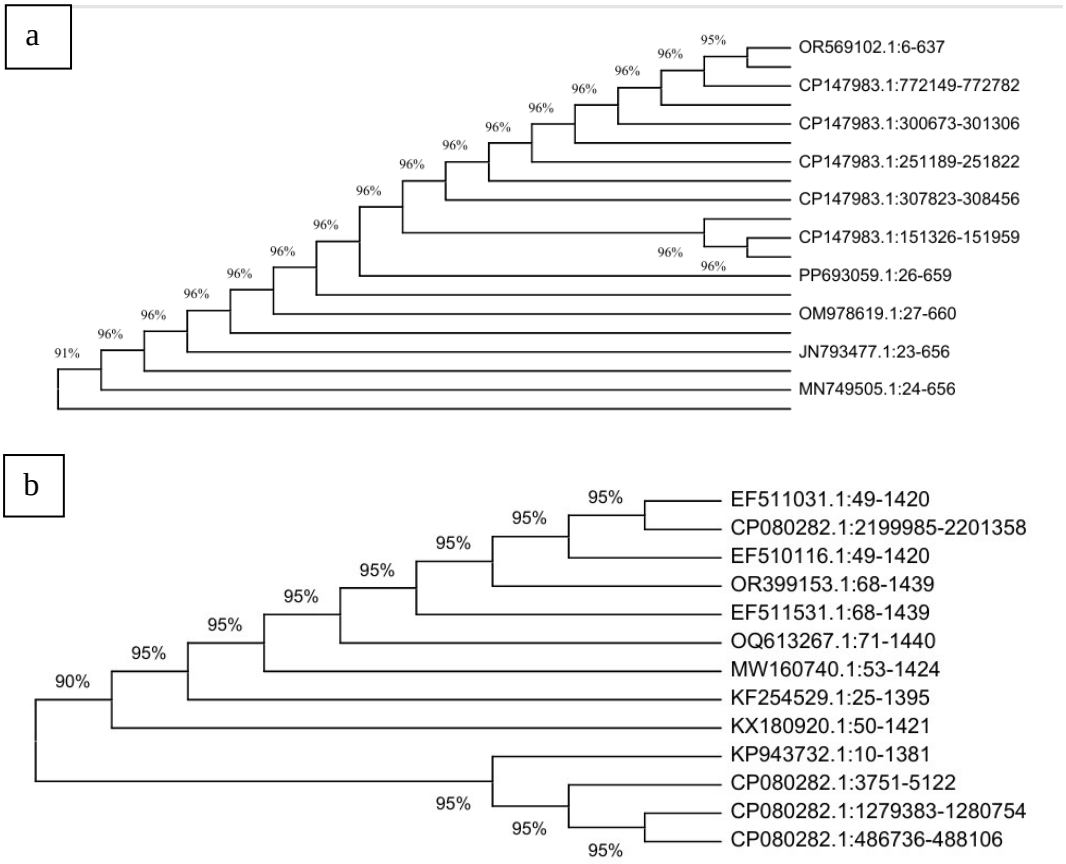


Fig 4. Evolutionary analysis by Maximum Likelihood method. (a) IS1: Identified as Bacillus paramycoides (b) IS2: Identified as Pseudomonas aeruginosa.

In the biodegradation assay, 10 mg polyethylene pieces were incubated in MSM with individual bacterial strains. Individual assays were conducted in triplicate (n=3) for *Bacillus paramycoides* and *Pseudomonas aeruginosa*. After 90 days of incubation, the polyethylene weight loss was 3.59 ± 0.01 % for *Bacillus paramycoides* and 4.85 ± 0.24 % for *Pseudomonas aeruginosa*, indicating the latter exhibited a higher biodegradation potential. One of the studies conducted found *Actinobacteria* and *Proteobacteria* to be LDPE degrading bacteria. The biodegradation rate of untreated and pretreated LDPE films was studied, and it was found out that the UV pretreatment of LDPE films increased the weight loss between 2.22 and 5.17%. In comparison, sunlight treatment led to 1.67-4.56% of weight loss, while the thermal treatment showed 1.42-3.22% weight loss and untreated LDPE had the lowest degradation of 1.32-2.80% in 120 days [33]. One study showed that *Cladosporium sphaerospermum* produced a weight loss of 15.23% in 7 days [34]. A study revealed that 47 fungal isolates belonging to 10 genera were obtained; out of them only 11 were able to grow

the LDPE film. While, after 90 days trial, only one isolate of *Cladosporium cladosporioides (Clc/1)* was able to carry out the degradation of LDPE film [35].

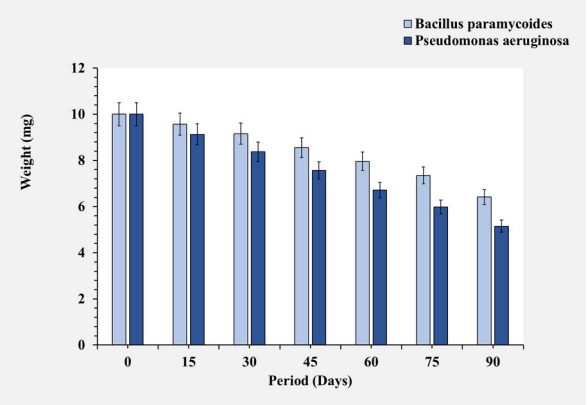
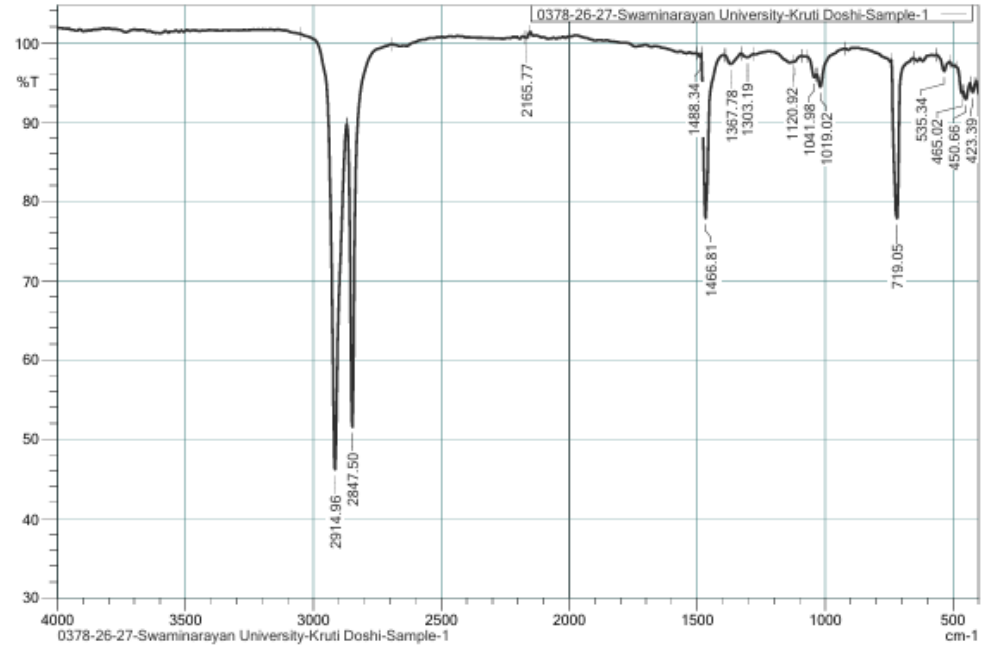


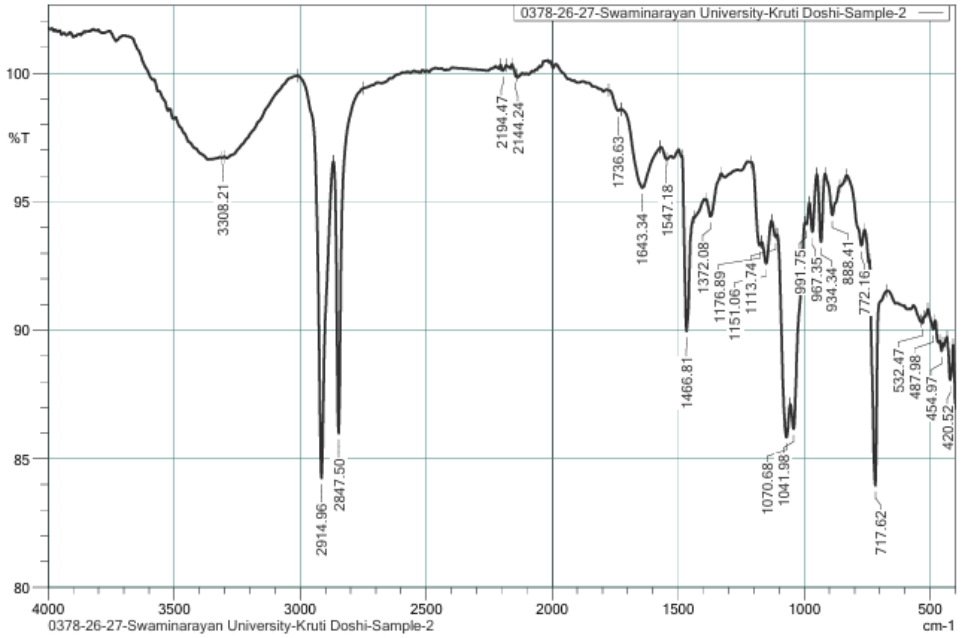
Fig 5. Biodegradation assay by weight loss study of IS1: Bacillus paramycoides (3.59%) and IS2: Pseudomonas aeruginosa (4.85 %) have ability to degrade polyethylene strips.

FTIR spectroscopic technique was used to study the chemical changes brought about by the microbes on the polyethylene films. Spectra were obtained for the range 4000-500 cm^{-1} and compared to those of the control to determine the effects of biodegradation on the functional groups. All treatments, including uninoculated controls containing MSM and polyethylene only, were set up in triplicate. Infrared Absorption Spectra of Polyethylene, for untreated polyethylene film (sample 1), the characteristic bands of the absorption spectra appeared at 2914.96 cm^{-1} and 2847.50 cm^{-1}, which were related to the asymmetric and symmetric vibration stretches of methylene (–CH_2–). Besides, the additional bands that appeared at 1466.81 cm^{-1} and 1367.78 cm^{-1} were related to the vibrations of CH_2 bend and CH_3 deformations, respectively. The band at 719.05 cm^{-1} was connected with CH_2 rocking vibrations for the crystalline area of polyethylene. These peaks confirmed the typical hydrocarbon structure of the polymer and served as reference bands for comparison with bacterial-treated samples. Exposure to *Pseudomonas aeruginosa* (Sample 2) led to the appearance of new bands assigned to hydroxyl (around 3308 cm^{-1}), carbonyl (around 1736 cm^{-1}), and other oxidized groups (around 1643 cm^{-1}), together with decreased intensity of the characteristic methylene stretching and bending bands. These changes indicate oxidative cleavage of the polyethylene backbone, reduced crystallinity, and formation of degradation intermediates. Also, the change in the intensity of absorption peaks at 2914.96 cm^{-1}, 2847.50 cm^{-1}, 1466.81 cm^{-1}, and 719.05 cm^{-1}, which are unique to polyethylene polymers, indicated destruction of the polymer chains and decrease in its crystallinity. The appearance of additional peaks in the fingerprint region (1151–934 cm^{-1}) further supports the formation of degradation intermediates resulting from microbial activity. Polyethylene treated with *Bacillus paramycoides* (Sample 3) also showed new peaks and reduced intensities of native bands, but the extent of spectral changes was less pronounced, consistent with its lower weight-loss values. The emergence of new peaks at 1643.34 cm^{-1} and 1515.61 cm^{-1} revealed the appearance of some kind of oxidation products and microorganism metabolites. Nevertheless, despite the presence of peaks typical for the polyethylene structure, namely 2914.96 cm^{-1}, 2847.50 cm^{-1}, 1466.81 cm^{-1}, and 719.05 cm^{-1}, their weakening indicated chain cleavage and the modification of polymer matrix. However, the extent of spectral changes was comparatively lower than that observed for *Pseudomonas aeruginosa*, indicating a relatively lower degradation efficiency. The comparative analysis of the IR spectra showed that in the case of bacterial treatments, there were formed oxygen-containing functional groups along with changes in the specific absorption bands of polyethylene. The presence of hydroxyl and carbonyl groups along with weakening of the absorption bands of stretching and bending of methylene groups showed oxidative destruction of polyethylene bonds. These gravimetric results are consistent with FTIR spectra, which showed

more pronounced formation of oxygen-containing functional groups in polyethylene films treated with *Pseudomonas. aeruginosa* than with *Bacillus paramycoides*, in which a higher percentage of polyethylene decomposition was observed for *Pseudomonas aeruginosa* (4.85 ± 0.24%) as compared to *Bacillus paramycoides* (3.59 ± 0.01 %) during 90 days of incubation period.

It was noted from one study that the result of ATR-FTIR analysis on the film with degradation revealed that there was a functional group added between 1025 and 1275 cm^{-1}, where the presence of C-O stretching increased as a result of adding alcohol (–OH) functional group, and this was confirmed by GC-MS analysis on fatty acid degradation by-products [25]. On the basis of another research, FTIR spectrum of LDPE film showed that there was a change in the presence of chemical groups such as amine, alkane, phenol, and alcohol in LDPE films degraded by *Aspergillus nomius* and *Streptomyces sp.* and the most significant change in the structure was seen in LDPE after 90 days of degradation [27].





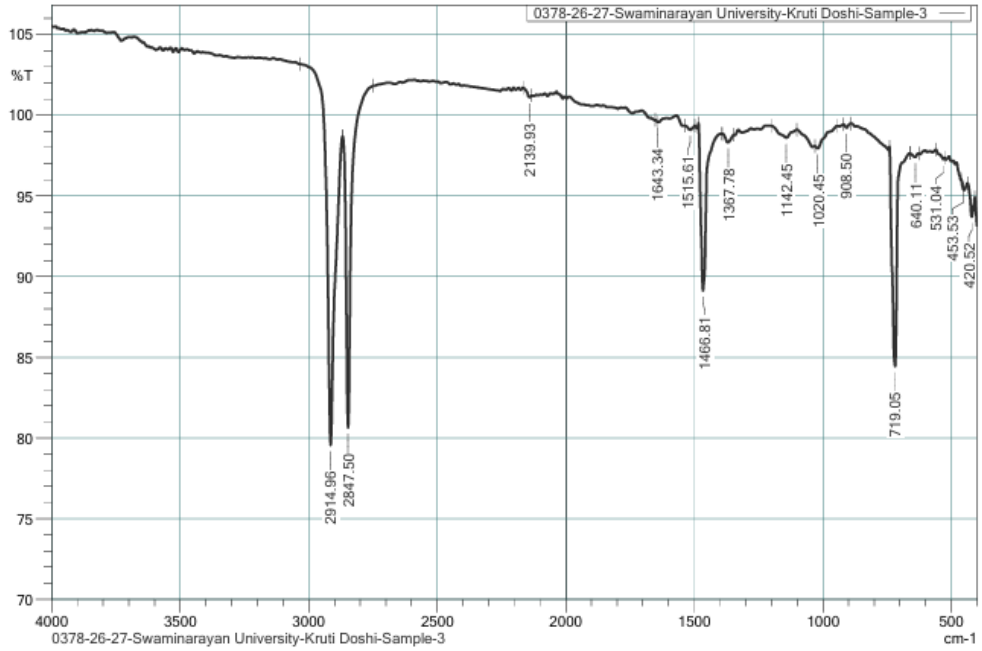


Fig. 6. FTIR spectra of polyethylene films before and after bacterial degradation. Sample 1: untreated polyethylene (control), Sample 2: polyethylene treated with Pseudomonas aeruginosa, and Sample 3: polyethylene treated with Bacillus paramycoides.

4 CONCLUSION

This research has been successful in isolating and characterizing polyethylene-degrading bacteria from soils of municipal dump sites in Kalol (Gandhinagar) and Pirana (Ahmedabad), Gujarat. Of the fifteen bacterial isolates which have been obtained using enrichment culture, only two bacteria were found to grow using polyethylene as the sole source of carbon in mineral salt medium. These bacteria have been characterized both morphologically and biochemically and their identity confirmed by rDNA analysis as *Bacillus paramycoides* and *Pseudomonas aeruginosa*.

Biodegradation test showed that both strains of bacteria had biodegradation capacity for polyethylene. During 90 days of incubation, *Pseudomonas aeruginosa* had higher biodegradation efficiency, where 4.85 ± 0.24 % weight reduction was obtained, while *Bacillus paramycoides* had a weight reduction of 3.59 ± 0.01 % of the polyethylene sample. Fourier Transform Infra-red (FTIR) analysis further validated the biodegradation process due to the presence of new functional groups produced during the biodegradation reaction. Polyethylene that underwent biodegradation test using *Pseudomonas aeruginosa* had more distinct modification, which proved higher biodegradation capacity compared to *Bacillus paramycoides*.

The results show that soil samples taken from municipal dumpsites serve as effective sources of local microbes that can degrade polyethylene films. *Pseudomonas aeruginosa* in particular exhibited significant potential for application in eco-friendly management of polyethylene waste. Future research efforts should focus on studying microbial communities, optimization of the degradation process, enzymatic analysis, and large-scale applications of these strains in the biodegradation of polyethylene films.

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