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Isolation, Screening and Evaluation of Low Density Polyethylene Degrading Bacteria from Plastic Contaminated Environments

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Abstract

Low-Density Polyethylene (LDPE) is one of the most abundantly produced synthetic polymers, and its non-biodegradable properties have led to a huge environmental buildup. The main objective of this study was to isolate microorganisms that can degrade LDPE from plastic contaminated sites using proven microbiological and laboratory techniques. Soil and water samples were obtained from sites where plastic was abundantly available, and microorganisms were isolated using conventional serial dilution and plating methods. The isolated microorganisms were tested for LDPE degradation on Bushnell-Hass medium containing LDPE as a sole carbon source on solid plate and liquid cultures as a part of screening. The microorganisms that can live in a carbon-deficient environment suggested that they are able to exist in a LDPE-rich environment. The alteration of the LDPE was analyzed using Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR-FTIR) Spectroscopy to demonstrate the chemical modification. Notable differences in the FTIR spectrum from those of the unaltered samples confirmed the interaction of the microbes with LDPE. Promising bacteria was characterized morphologically, culturally and biochemically and analyzed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), a common method for the identification of microorganisms. The significance of this study lies in the potential of the indigenous microorganisms in the biodegradation of LDPE.

Keywords: LDPE degradation, Plastic contaminated sites, Bushnell–Hass medium, ATR-FTIR analysis, MALDI-TOF MS

Introduction

The use of synthetic plastics in large quantities makes them vital materials in today's world due to their toughness, flexibility and cost-effectiveness. Some of these include low-density polyethylene (LDPE), which is among the most widely produced thermoplastics and which has a wide range of applications including packaging films, shopping bags, agricultural mulch films, bottles and many other disposable

goods. Nevertheless, due to the high molecular weight, hydrophobicity and the chemical stability of the carbon chain of the LDPE plastic, the material takes a very long time to decompose. This has made LDPE waste accumulation becoming a very serious environmental problem in both land and water environments leading to environmental pollution, harm to fauna and formation of microplastics (Geyer et al., 2017; Jambeck et al., 2015; Restrepo-Flórez et al., 2014).

Plastic Waste Management practices such as landfilling, incineration and mechanical recycling of polyethylene waste face considerable economic and environmental challenges. Landfilling consumes precious land while incineration creates emissions that are both environmentally detrimental and toxic. Although recycling is a viable waste management practice, its efficacy is hampered by contamination, improper segregation and degradation of polymer. Therefore, there has been increasing focus on finding environmentally sustainable ways of managing polyethylene waste. One of the methods that have been explored is microbial degradation since microbes can inhabit polyethylene surfaces, create biofilm and secrete extracellular enzymes to facilitate oxidation and depolymerization of the polymer into smaller units that may later be utilized as carbon sources (Arutchelvi et al., 2008; Mohanan et al., 2020; Ru et al., 2020).

Plastic-contaminated environments like landfills, municipal dumping sites, plastic contaminated soil, water bodies are considered ideal sources for the isolation of microorganisms capable of polyethylene degradation. Long-term exposure to plastic waste could enable certain bacteria or fungi to adapt to the plastic and use polyethylene or degradation intermediates as a carbon source in limiting conditions. Thus, the screening of microorganisms from these environmental sources is one of the ways to find polyethylene degraders. Various analytical methods, including Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy, are routinely used to study structural modifications of polyethylene after microbial treatment through detecting changes in the presence of characteristic functional groups. Moreover, putative identification of effective degraders through sophisticated tools such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a useful tool (Montazer et al., 2019a; Pathak & Navneet, 2017).

Given the rising environmental load due to polyethylene waste pollution, the objective of this research was to screen out indigenous microorganisms from polyethylene contaminated soil and water samples and assess their capability to degrade LDPE. Microbial strains were screened under conditions of growth on Bushnell-Haas medium containing LDPE as the sole source of carbon and chemical changes in the polymer were studied through ATR-FTIR spectroscopy. Selected bacteria were identified using various morphological, cultural and biochemical tests and MALDI-TOF MS analysis. The results from this study help in identifying indigenous LDPE-degrading microorganisms.

Materials and Methods

Sample Collection

Soil and water samples were collected from various plastic contaminated sites from Surat, Gujrat, India. The samples were collected aseptically in zip-lock bags and transported to the laboratory under chilled conditions. The samples were stored at 4 °C until further processing. The samples were processed for measurement of their pH values before any other processing.

Enrichment of Samples

One gram of soil or 1 mL of water sample was aseptically suspended in 9 mL of sterile distilled water and allowed to settle. Later, 1% (v/v) inoculum was transferred into Bushnell & Hass (BH) medium with 1%

LDPE powder as the only carbon source. Cultivation was done at 30°C with agitation speed at 120 rpm for 30 days.

At every one-week interval, 0.5 mL of the culture broth was withdrawn aseptically and serially diluted up to 10^{-6} and plated onto Nutrient Agar plates (Kim et al., 2025). Enumeration in terms of CFUs was done after incubation of the agar plates. The results showed a gradual reduction in the number of CFUs with each subsequent week, suggesting that selective pressure was exerted by the polyethylene (LDPE) medium (Salinas et al., 2023; Salini et al., 2024). Microorganisms which could resist these conditions were isolated and purified for subsequent screening.

Screening of LDPE Degrading Microorganisms

Solid as well liquid medium techniques were used to carry out screening for potential LDPE degrading isolates from plastic contaminated sites.

Primary Screening of LDPE Degrading Microorrganisms

Initially, the primary screening was carried out by using polyethylene glycol (PEG) (Rana & Rana, 2020), n-hexadecane and LDPE as a sole source of carbon. Nutrient agar and Bushnell Hass agar media supplemented with 1% PEG were inoculated with the pure cultures and incubated for 14 days at 30 °C and BHA supplemented with 1.0% n-hexadecane and 0.1% LDPE (Kannahi & Sudha, 2013) were inoculated and incubated for 7 days at 30 °C (Montazer et al., 2019a). Only those isolates that could degrade PEG, n-hexadecane and LDPE showed signs of growth, thus excluding the non-degraders.

Secondary Screening of LDPE Degrading Microorganisms

Secondary screening was carried out in Bushnell-Hass broth with 1% LDPE powder as the only carbon source. The isolates were inoculated in 1% concentration with an initial optical density of 1.0 at 600 nm (2×10^8 cells/mL of bacteria, 2×10^8 spores/mL of fungi & actinomycetes). The incubation period was 30 days at 30°C & 120 rpm. The uninoculated media with LDPE acted as an abiotic control & Bushnell-Hass media without LDPE acted as the negative control. Bacterial growth was determined weekly by measuring optical density and cell viability.

After 30 days, LDPE powder was extracted by Whatman filter paper with a pore size of 11 µm, cleaned with a 2% SDS solution and then finally rinsed with sterile distilled water. Finally, the extracted polyethylene powder was dried at a temperature of 60°C for a period of 4 hours and the reduction in weight measurements were taken to confirm degradation, further confirmed by FTIR analysis.

Confirmation of LDPE Degradation by ATR-FTIR Analysis

The chemical modification of LDPE resulting from incubation with isolates was assessed using Attenuated Total Reflectance-Fourier Transform-Infrared (ATR-FTIR) spectroscopy (PerkinElmer GX, USA), according to standard procedures (Tareen et al., 2022). The scanning range of 4000-400 cm^{-1} was used in order to record 45 scan average spectra.

Isolation and Characterization of Isolates

Morphological Characterization of Isolates

Bacterial and actinomyces cultures were stained with Gram staining, whereas fungal morphology was observed using mounting with lactophenol cotton blue stain. Cultural characteristics were observed under nutrient agar for bacteria and actinomyces, and potato dextrose agar media for fungi (Dey et al., 2020).

Biochemical Characterization of Isolates

Four bacterial isolates were evaluated in the carbohydrate fermentation (glucose, maltose, xylose, sucrose, mannitol and lactose) test reactions, IMViC series of tests, nitrate test, triple sugar iron agar test, gelatin

hydrolysis test, urease test and hydrogen sulfide production test.

Identification of Isolate BKAGSE6 using MALDI-TOF MS

Pure colony was deposited as spot on a MALDI target plate, overlaid with matrix solution and then analyzed by MALDI-TOF MS/MS. Identification was carried out by protein spectral fingerprints by matching with standard libraries. Results were interpreted as per similarity scores, divided into strong, weak or irrelevant hits (Rahi et al., 2016).

Results

Isolation of LDPE Degrading Microorganisms

A total of 54 microorganisms were isolated after the enrichment procedure of 30 days by serial dilution and plating. The isolate includes 42 bacteria, 9 actinomycetes and 3 fungi based on the morphological and cultural characteristics. The colonies were purified and maintained on NA and PDA slants at 4°C, while glycerol stocks were stored at −20°C (Alharbi et al., 2026).

Primary Screening of Microorganisms

Only those isolates that could degrade PEG, n-hexadecane and LDPE showed signs of growth, thus excluding the non-degraders. After the primary screening, 6 strains (3 bacteria, 2 actinomycetes and 1 fungus) were chosen for the secondary screening test.

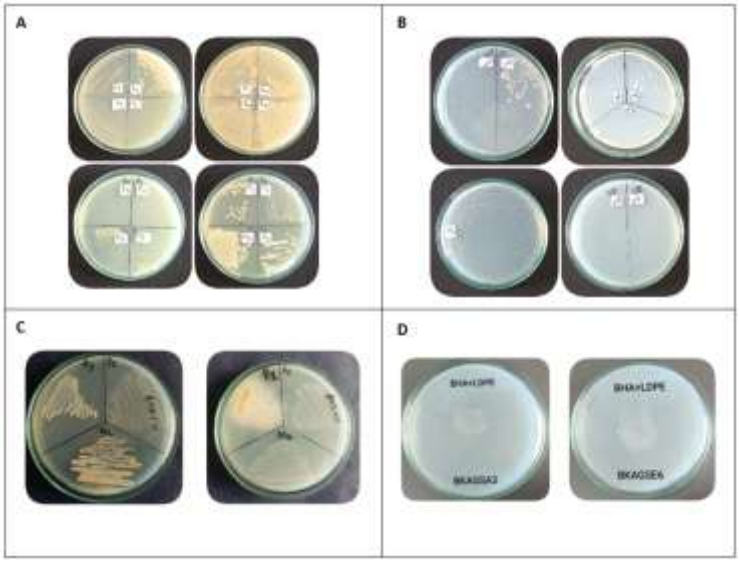

Figure 1: Primary Screening of Isolates on (A) NA + 1% PEG, (B) BHA + 1% PEG, (C) BHA + 1% n-Hexadecane and (D) BHA + 0.1% LDPE

Secondary Screening of LDPE Degrading Microorganisms

The isolates showing positive results from the primary screening experiments were subjected to secondary screening experiment in Bushnell-Haas broth medium supplemented with 1% LDPE powder as the only carbon source for 30 days. All the chosen isolates survived and grew when limited with carbon sources, hence, showing the ability to consume LDPE or its degradation products. Nevertheless, the level of growth and biodegradation ability was different between the isolates.

Gravimetric measurement of LDPE powder after incubation showed that there were differences in weight loss of the isolates used. From the results of the screening experiments, the isolate BKAGSE6 showed high biodegradation potential of LDPE (6.4 % observed weight loss) and thus, chosen for further

evaluation. The biodegradation capability of the isolate was further confirmed through changes in the characteristic absorption peaks of LDPE by the ATR-FTIR spectra of the treated LDPE sample.

The ATR-FTIR-Based Confirmation of LDPE Degradation

ATR-FTIR spectra showed clear differences of LDPE exposed to the chosen isolate (BKAGSE6) compared to the abiotic control and standard samples. These variations in the region of C–H bonds, together with the appearance or intensification of bands related to the carbonyl bonds (1047 cm^{-1}), confirm the oxidative degradation of the polymer chains. These variations in the FTIR spectrum have been universally recognized as signs of degradation of the polymer.

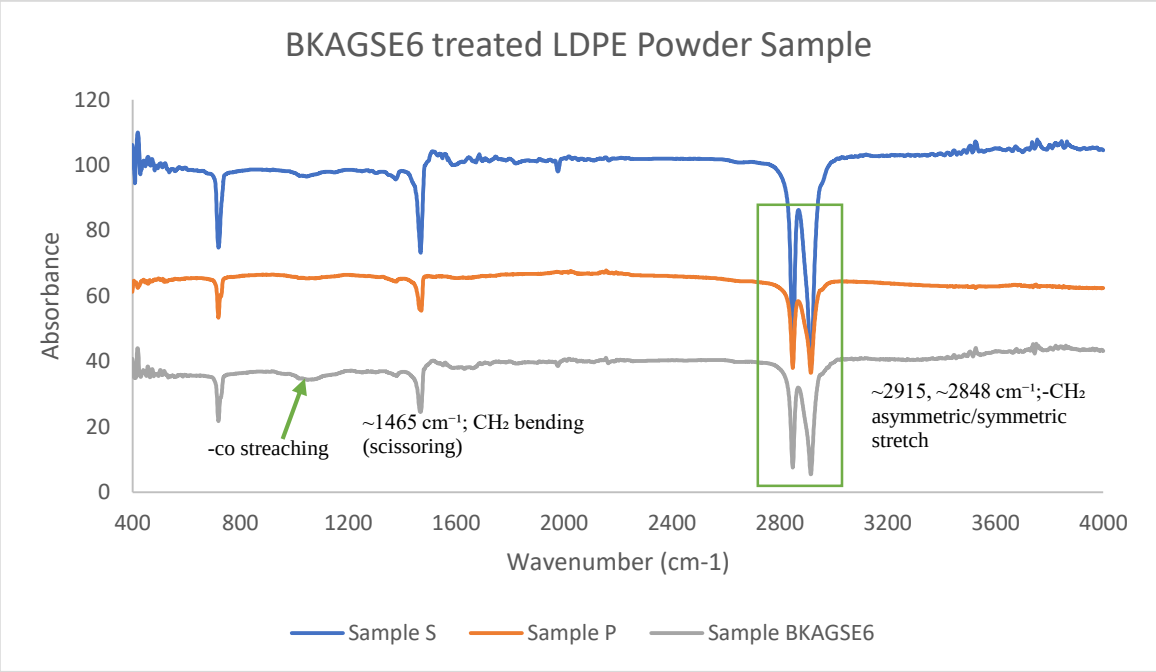

Figure 2: ATR-FTIR spectra of LDPE powder sample after 30 days study with Sample S(Standard) and Sample P (Abiotic Control)

Selection of Potential Isolate

Based on the results of primary and secondary screening and ATR-FTIR characterization isolate BKAGSE6 found to be potential LDPE degrader. It shows good growth even after 30 days in carbon-deficient liquid medium having LDPE as a sole source of carbon.

Identification of Isolate BKAGSE6

Morphological, Cultual and Biochemical Characterization of Isolate BKAGSE6

Morphological, cultural and biochemical characteristics of the isolate BKAGSE6 are provided in Table 1.

Characters	BKAGSE6	Isolate	BKAGSE6
Size	Large	1. Carbohydrate Utilization Test	
Shape	Irregular	Glucose	A+
Margin	Undulate	Lactose	-
Elevation	Umbonate	Maltose	-

Texture	Rough	Mannitol	A+
Consistency	Dry	Sucrose	A+
Opacity	Opaque	Xylose	A+
Pigment	No	2. Methyl Red Test	-
Gram Reaction	Positive	3. Vogues Proskauer Test	+
Shape in Microscopy	Rods	4. Indole Production Test	-
Motility	Non-motile	5. Simmon Citrate Test	+
		6. Urea Hydrolysis Test	+
		7. Gelatin utilization Test	+
		8. Nitrate Reduction Test	+
		9. Hydrogen Sulphide Production Test	-
		10. Tripple Sugar Iron Utilization Test	Butt acidic, Slant alkaline

Table 1: Morphological, Cultural and Biochemical Characteristics of Isolate BKAGSE6

The isolate formed irregular-shaped colonies which were characterized to be dry in nature and possessed Gram-positive and rod-shaped cells. Phenotypic analysis of the isolate showed that the tests such as citrate utilization, urea production, gelation hydrolysis and nitrate reduction were positive while others gave variable results. Overall, the isolate had similarities with the members of the genus *Bacillus*.

Identification of isolate BKAGSE6 by MALDI-TOF MS

MALDI-TOF MS analysis was performed to determine the taxonomic identity of isolate BKAGSE6 based on its protein spectral fingerprint. The obtained mass spectrum showed the closest match with *Bacillus licheniformis*. However, the identification was associated with a low-confidence (yellow) similarity score, indicating a putative species-level assignment. Therefore, isolate BKAGSE6 was tentatively identified as *Bacillus licheniformis* and was subjected to further molecular characterization for confirmation.

Rank (Quality)	**Matched Pattern**	**Score Value**	**NCBI Identifier**
1 (+)	*Bacillus licheniformis* CS 54 1 BRB	**1.97**	1402
2 (+)	*Bacillus licheniformis* DSM 13T DSM	**1.95**	1402
3 (+)	*Bacillus licheniformis* 992000432 LBK	**1.86**	1402
4 (+)	*Bacillus licheniformis* CICC 23972 CICC	**1.70**	1402
5 (–)	*Bacillus sonorensis* DSM 13779T DSM	**1.63**	119858
6 (–)	*Comamonas aquatica* LMG 2370T HAM	**1.51**	225991

7 (–)	*Bacillus licheniformis* DSM 394 DSM	**1.47**	1402
8 (–)	*Bacillus licheniformis* DSM 28096 DSM	**1.40**	1402
9 (–)	*Stenotrophomonas acidaminiphila* DSM 13117T HAM	**1.30**	128780
10 (–)	*Bacillus licheniformis* DSM 11258 DSM	**1.29**	1402

Table 2: MALDI-TOF MS identification results for isolate BKAGSE6 showing the top ten database matches based on protein spectral fingerprint analysis

Discussion

Plastic-polluted ecosystems have been viewed as favorable sources for polyethylene-degrading microbes due to the fact that long-term exposure to plastic wastes leads to the selection of microorganisms that can degrade non-soluble carbon. In the current experiment, enrichment and selective screening yielded a total number of 54 microorganisms, out of which only 6 could grow in PEG, n-hexadecane and LDPE, suggesting their capacity for hydrocarbon utilization. Additionally, the screening process conducted in Bushnell-Haas medium with LDPE as the only carbon source showed that all isolates could survive in the presence of limited amount of carbon, albeit their efficiency in biodegradation differed. Among these, BKAGSE6 had the highest potential for LDPE degradation, based on gravimetric assay, and thus it was chosen for further experiments. Successful isolation of polyethylene degraders through such screening procedures has been reported earlier (Montazer et al., 2019b; Restrepo-Flórez et al., 2014).

The biodegradation capacity of the BKAGSE6 strain was also confirmed by analyzing the shifts in the characteristic absorption peaks of LDPE via ATR-FTIR technique. Modification in the C-H stretching zone along with formation or enhanced intensity of the carbonyl groups implies the oxidative attack on the polymer, which is a vital early stage of biodegradation of the polyethylene plastic. The changes in chemical composition enhance the susceptibility of the polymer to be attacked by microorganisms, and they have been extensively used as proof of polyethylene degradation by bacteria (Arutchelvi et al., 2008; Ojha et al., 2017; Ru et al., 2020). However, while ATR-FTIR cannot prove the complete mineralization of LDPE, the detected changes, together with weight loss, are good proof of biological action on the polymer.

From morphological, cultural, biochemical and MALDI-TOF MS analysis, isolate BKAGSE6 displayed features typical of the genus *Bacillus*, with the most similar protein spectral profile belonging to *Bacillus licheniformis*. The putative identification of the isolate was made because of the low-confidence score in the identification using MALDI-TOF MS technique. However, *Bacillus* species have been described for their environmental adaptation, ability to form biofilms and secretion of extracellular enzymes linked with degrading hydrocarbons and polyethylenes (Mohanan et al., 2020; Pathak & Navneet, 2017). This makes the isolation of BKAGSE6 strain from a polluted site together with its ability to alter LDPE an interesting prospect in studying polyethylene biodegradation.

Conclusion

In this study, indigenous microorganisms capable of degrading low density polyethylene (LDPE) have been isolated and characterized from contaminated environments. Out of all the isolates, the strain BKAGSE6 was found to be the most efficient biodegrader of LDPE through secondary screening in Bushnell-Haas medium using LDPE as the only carbon source. The gravimetric analysis and ATR-FTIR spectra revealed the alteration in the polymer due to microbial actions and thus signifying the beginning of degradation of LDPE. The morphological, cultural, biochemical and MALDI-TOF MS studies

suggested the isolate to be *Bacillus licheniformis*. The results showed that indigenous microorganisms from plastic contaminated environments could be used as biodegraders of polyethylene and provide a basis for further molecular characterization and investigation of their potential applications in sustainable plastic waste management.

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