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Comparative Biodegradation of HDPE and LDPE Using an Indigenously Developed Microbial Consortium

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A variety of bacterial strains were isolated from waste disposal sites of Uttaranchal, India, and some from artificially developed soil beds containing maleic anhydride, glucose, and small pieces of polyethylene. Primary screening of isolates was done based on their ability to utilize high- and low-density polyethylenes (HDPE/LDPE) as a primary carbon source. Thereafter, a consortium was developed using potential strains. Furthermore, a biodegradation assay was carried out in 500-ml flasks containing minimal broth (250 ml) and HDPE/LDPE at 5 mg/ml concentration. After incubation for two weeks, degraded samples were recovered through filtration and subsequent evaporation. Fourier transform infrared spectroscopy (FTIR) and simultaneous thermogravimetric-differential thermogravimetry-differential thermal analysis (TG-DTG-DTA) were used to analyze these samples. Results showed that consortium-treated HDPE (considered to be more inert relative to LDPE) was degraded to a greater extent (22.41% weight loss) in comparison with LDPE (21.70% weight loss), whereas, in the case of untreated samples, weight loss was more for LDPE than HDPE (4.5% and 2.5%, respectively) at 400°C. Therefore, this study suggests that polyethylene could be degraded by utilizing microbial consortia in an eco-friendly manner.

Keywords: HDPE, LDPE, biodegradation, thermal analysis, Fourier transform infrared spectroscopy, consortium

Plastic materials are strong, light-weight, and durable and thus are widely used in food, clothing, shelter, transportation, construction, medical, and recreation industries. However, because of its xenobiotic origin and recalcitrant nature, its biodegradation is problematic and it accumulates at a rate of 25 million tons per year [6]. Polyethylene is one of the most inert plastic materials and its recalcitrant nature results from its high molecular weight, complex three-dimensional structure, and hydrophobic nature [5], all of which interfere with its availability to microorganisms. It can be classified as high-density and low-density polyethylenes (HDPE and LDPE). LDPE is characterized by good toughness, resistance to chemicals, flexibility, and clarity, whereas HDPE is more rigid, harder, and has a greater tensile strength than that of LDPE. Despite of their wide applicability, the main limitation to their use is the fact that polyethylene adversely affects the environment.

Thus, to deal with this environmental menace, biodegradation appears to be the best choice, as the other two approaches, land filling and incineration, have their own limitations. However, earlier reports have shown that no signs of deterioration could be observed in polyethylene sheet incubated in moist soil for 12 years [8], and only partial degradation was observed in a polyethylene film buried in soil for 32 years [7]. However, according to some reports, partial biodegradation of polyethylene could be achieved after UV irradiation [3], thermal treatment [1, 9], and/or oxidation with nitric acid [2].

In the present paper, a comparative biodegradation study of HDPE and LDPE is described, using an indigenously developed bacterial consortium. The consortium was developed on the basis of screening of individual strains to utilize polyethylene as a primary carbon source. Furthermore, individual bacterial strains have been characterized by their 16s rDNA sequencing and their sequences have been submitted to the NCBI database.

Materials and Methods

Polyethylene

High-density polyethylene (HDPE) and low-density polyethylene (LDPE; purchased from Sigma-Aldrich Chemical Corporation, U.S.A.) beads were converted into powdered form through boiling with xylene followed by solvent evaporation at room temperature.

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Table 1. Isolation profile of the cultures used in this study (PN, RGR, CN, CNA, CP, LNR are the isolates' names based on their isolation site).

Isolation sites	Strains
Artificial soil bed + polyethylene pieces, Pantnagar (India)	PN11, PN12, PN13, PN14, PN15
Artificial soil bed + polyethylene pieces + 0.5% glucose + 0.3% maleic anhydride, Pantnagar (India)	PN21, PN22, PN23, PN24, PN25, PN26
Dolichos lablab rhizosphere, Ranichauri (India)	RGR7, RGR10
Soil sample, Chamoli (India)	CN4, CNA2, CP1
Soil sample, Leh (India)	LNR3

The powdered HDPE/LDPE were successively washed with ethanol and used for biodegradation studies as a primary carbon source. The polyethylene beads were powdered so as to increase the surface area and to allow greater degradative action of the consortium.

Screening of Bacterial Isolates and Culture Conditions

A total of 17 bacterial strains selected for this study were obtained from a departmental culture collection and were originally isolated from different plastic waste disposal sites and artificial soil beds (Table 1). These isolates were further screened for HDPE/LDPE utilization as a primary carbon source. On this basis, one culture from each selected site was taken for further studies.

For the purpose of screening, active culture of isolate(s) was prepared in nutrient broth, and after 24 h incubation with continuous shaking (120 rpm) at 37°C, an aliquot was extracted and added to minimal broth (Davis w/o dextrose, Himedia Laboratories Pvt. Ltd., India) containing (g/l of distilled water) dipotassium phosphate, 7.0; monopotassium phosphate, 2.0; sodium citrate, 0.50; magnesium sulfate, 0.10; ammonium sulfate, 1.0; in addition, HDPE/LDPE was also added at a concentration of 5 mg/ml. After incubation at 37°C for 4 days, OD was taken at 600 nm using a spectrophotometer (Perkin Elmer, Lambda 35).

16s rDNA Sequencing and Phylogenetic Studies

Based on the screening, three potential strains PN12, PN24, and LNR3 were selected for biodegradation studies and were sent to the National Centre for Cell Sciences (NCCS), Pune, India, for their molecular characterization based on 16s rDNA sequencing. Polymerase chain reaction (PCR) amplification of almost the full-length 16S rRNA gene was carried out with the bacterial primer set 16F27 (5'-CCAGAGTTTGATCMTGGCTCAG-3') and 16R1525XP (5'-TTC-TGCAGTCTAGAAGGAGGTGWTCCAGGC-3'). PCR was performed in an automated gene amplification PCR system 9700 thermal cycler (Applied Biosystem, Foster City, U.S.A.), and the PCR product was sequenced using a BigDye terminator cycle sequencing kit (V3.1) in an ABI Prism 3730 Genetic Analyzer (Applied Biosystem, U.S.A.) using primer 16F27 to yield a 700-base 5' end sequence. The sequence was then analyzed at the RD11 and NCBI database. All the sequences were submitted to the NCBI database to get accession numbers. The phylogenetic tree was constructed using the neighbor-joining tree method and the phylogenetic data were obtained by aligning the different sequences of the 16s rRNA gene of closely related (99% DNA identity) bacteria using the BLAST search tool (National Center for Biotechnology Information [http:// www.ncbi.nlm.nih.gov]).

Development of Consortium

The three potential strains selected above were used for consortium development based on preliminary nutritional screening. A single colony from each culture was inoculated in 20-ml test tubes containing 5 ml of nutrient broth (pH 7.0±0.2), and the tubes were incubated at 37°C for 10 h with continuous shaking at 120 rpm. The calculated amount (CFU/ml) of each strain was mixed for development of the consortium. However, the exact amount is not disclosed because of patent issues.

Biodegradation Assay

The biodegradation assay was performed in 500-ml flasks by adding 150 µl of active consortium into 250 ml of minimal broth containing HDPE/LDPE each at a concentration of 5 mg/ml. The assay was performed with respective positive (minimal broth+consortia) and negative (minimal broth+ HDPE/LDPE) controls. The flasks were incubated at 37°C with continuous shaking (120 rpm). After the consortium had attained its stationary growth phase, degraded samples were recovered from the broth.

Recovery of Degraded Product

The degraded compound was recovered from the broth after filtration and subsequent evaporation of the filtrate. Centrifugation of the filtrate was done at 2,348 ×*g* (5,000 rpm) for 15 min to remove bacterial biomass. The supernatant was kept in an oven at 60°C for overnight to evaporate water, and the residual sample was recovered and subsequently analyzed by Fourier transform infrared (FITR) spectroscopy and simultaneous thermogravimetric-differential thermogravimetry-differential thermal analysis (TG-DTG-DTA).

FTIR Spectroscopy and TG-DTG-DTA

Samples were analyzed by FTIR, and different peaks relative to CH_2 deformation, CH_2 bending (symmetrical), CH_2 bending (asymmetrical), CH_2 stretching, CH stretching, and C-O bond were compared taking pure HDPE/LDPE as a reference.

TG-DIG-DTA was done on a TG analyzer (Perkin Elmer, Pyris Diamond) from 20°C to 550°C under a nitrogen atmosphere (400 ml/min) using a heating ramp of 5°C/min in a platinum pan.

Results and Discussion

Isolation of Bacterial Isolates

As reported earlier [4], biodegradation of plastics can be accelerated up to 30% by addition of sugar in the form of glucose and maleic anhydride, hence artificial soil beds were prepared containing 0.5% glucose, 0.3% maleic anhydride, and small polyethylene pieces. This soil bed was left for three months to allow indigenous microorganisms

Table 2. Accession numbers of 16s rRNA sequences of three strains submitted to the NCBI database.

Strain No.	Strain	Accession No.
1	PN24	DQ423485
2	PN12	DQ423487
3	LNR3	DQ486130

to adapt to this environment and to enrich polyethylene degrading microflora. This was done in view of the fact that adaptation also plays a major role in determining biodegradation rates. Furthermore, proper moisture and aeration conditions were maintained by adding 500 ml of water and shoveling the soil on each alternate day.

Thus, the bacteria isolated from such soil beds are supposed to have some degradation activity for polymers present in their microenvironment. The remaining cultures were isolated from polyethylene waste disposal sites of Chamoli (India), Leh (Ladakh), and from rhizospheric soil of Gahat (*Dolichos lablab*), respectively.

Characterization of Potential Strains and Phylogenetic Analysis

Analysis of their partial 16s rRNA sequences has shown that strain PN24 is closely related (99% DNA identity) to *Bacillus cereus* species (accession numbers AJ577289 and AJ577290), PN12 has 99% DNA identity with the 16s rRNA sequence of *Bacillus pumilus* species (accession numbers EF491624 and EF488975), and LNR3 has 99% DNA identity with that of *Arthrobacter* species (accession numbers AJ243422 and DQ157996). The partial 16s rRNA sequences from the three polyethylene degraders have been deposited in the NCBI GenBank database under the accession numbers indicated in Table 2. The phylogenetic analysis of the partial 16s rRNA gene sequences of the three strains (PN24, PN12, and LNR3) is shown in Fig. 1. All three strains belongs to three different clusters: PN24 formed a cluster with *Bacillus cereus* species, PN12 formed a cluster with *Bacillus pumilus* species, and LNR3 formed a cluster with *Arthrobacter* species.

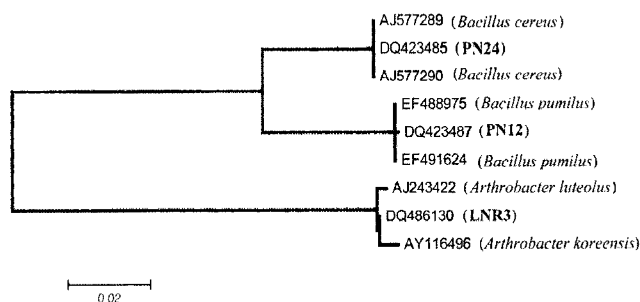

Fig. 1. Phylogenetic tree of polyethylene-degrading strains based on the sequences of 16s rRNA constructed with the neighbor-joining method.

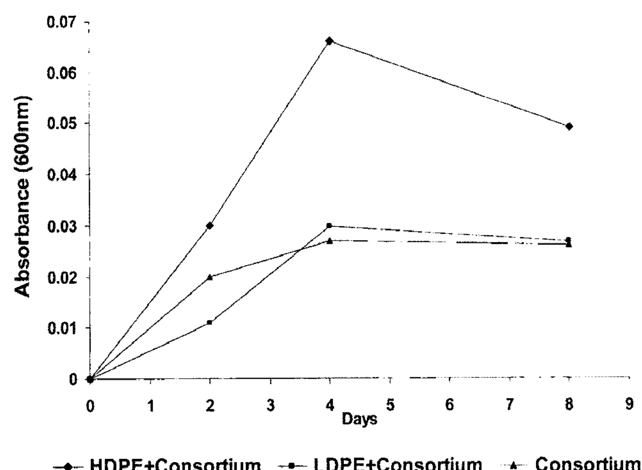

Fig. 2. Growth profile of consortia in the presence and absence of HDPE and/or LDPE.

Comparative Growth Profile of Consortium in Presence/Absence of HDPE/LDPE

Consortia reached to the stationary phase at the 6th day of incubation in the presence or absence of HDPE/LDPE. This shows that there is no adverse effect on the consortium growth by HDPE/LDPE (Fig. 2). In the presence of HDPE, the growth of the consortium was better than its counterpart (LDPE); however, the stationary phase was achieved at a similar time (8 days, Fig. 2) and higher than

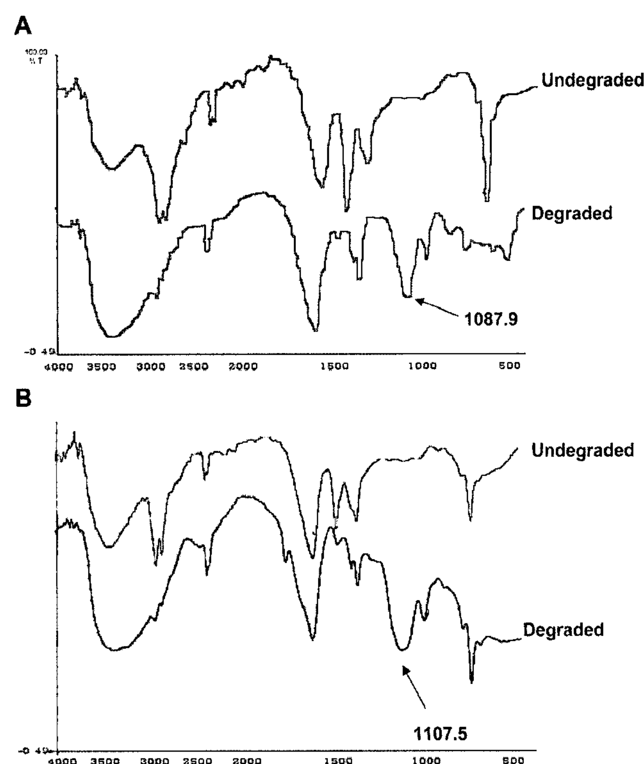

Fig. 3. FTIR spectra depicting *in vitro* (A) HDPE biodegradation and (B), LDPE biodegradation by the consortium over ten days.

Table 3. Comparative DTG and DTA of undegraded and degraded HDPE and/or LDPE samples.

Sample name	Consortia	DTG peak temp		DTA exotherm		Endotherm	
		Temp (°C)	Rate (mg/min)	Temp (°C)	mJ/mg	Temp (°C)	mJ/mg
HDPE	-	454	2.0	–	–	123	153
						55	109
LDPE	-	451	1.79	467	53.0	107	129
						457	14.6
HDPE	+	39	0.1	–	-		
		131	0.2			40	8.77
		141	0.2			133	70.1
		250	0.0			196	38.3
LDPE	+	43	0.09	–	–	44	42.7
		177	0.02	–	–	118	6.66
		438	0.04			176	17.1
		527	0.026				

the other two cases, showing that the consortium grew relatively better in its presence.

FIIR Spectra

Comparative FTIR spectra of biodegraded samples of HDPE and LDPE showed common absorption frequencies corresponding to CH stretching at 3,389.4–3,402.1 cm^{-1}, CH_2 deformations of 1,595.9–1,599.6 cm^{-1}, CH_2 bending (asymmetrical) at 1,460.0–1,464.0 cm^{-1}, and CH_2 bending (symmetrical) at 1,351.2–1,351.3 cm^{-1} (Fig. 3). A significant shifting in CH_2 stretching and C-O stretching frequencies was observed, due to microbial degradation of HDPE and

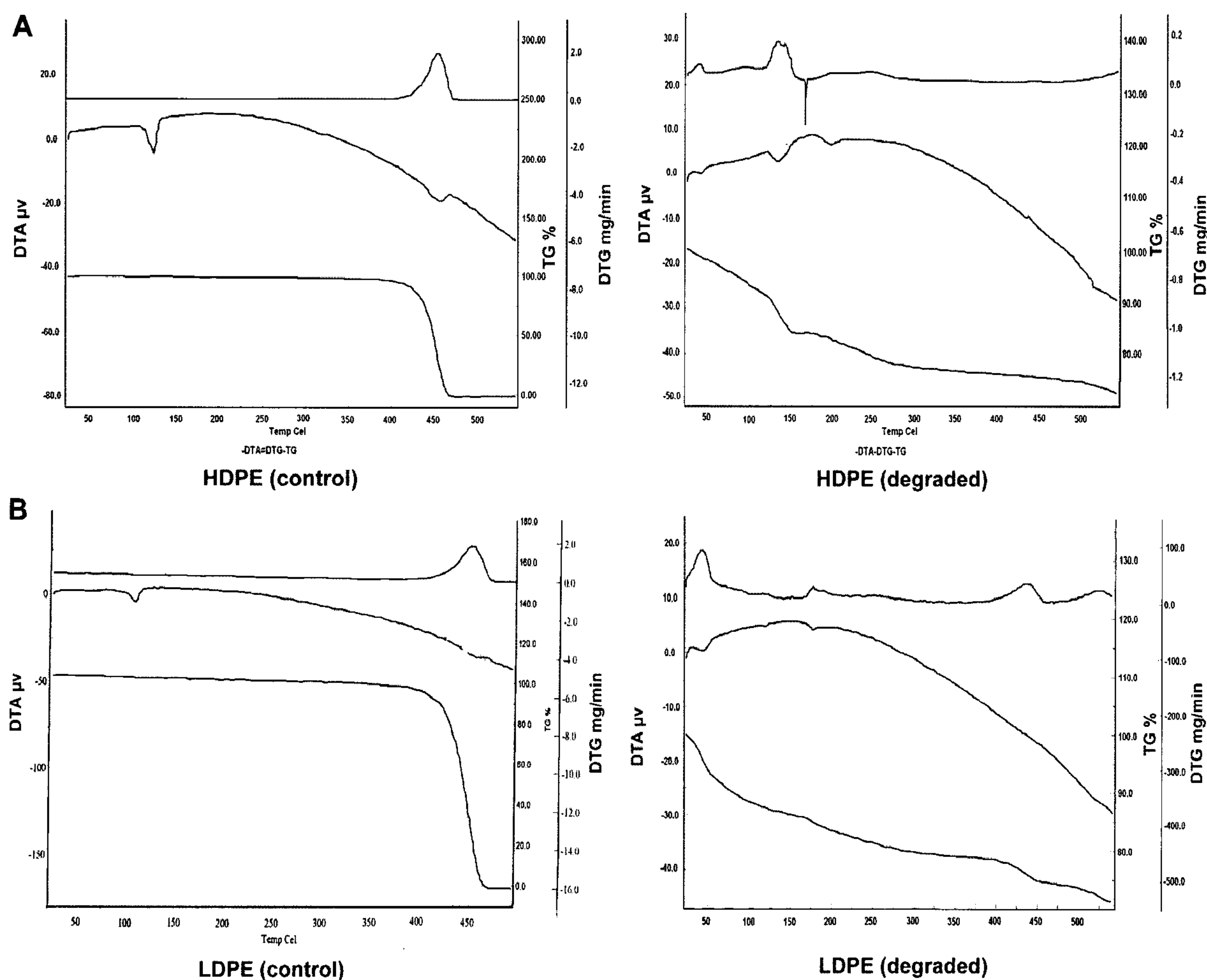

Fig. 4. Thermal analysis of degraded (**A**) HDPE and (**B**) LDPE samples with reference to control.

LDPE. Pure HDPE and LDPE (controls) did not show any C-O stretching frequency, whereas its biodegradation in the presence of the consortium showed C-O stretching frequencies ranging from 1,087.9–1,107.5 cm^{-1}. These observations clearly indicate that the consortium had significantly affected the carbon chain of LDPE/HDPE and induced bioactive hydrolysis.

Simultaneous TG-DTG-DTA

As TG analysis accurately measures the changes in the weight of a given compound at defined temperatures and time intervals, it allows us to determine the thermal stability of the compound. The weight reduction rate under defined conditions is inversely proportional to the size of the polymeric chains. Thermal data of the consortium-degraded HDPE and LDPE samples with reference to controls have been summarized in Table 3.

TGA thermograms obtained for pure HDPE and its biodegraded sample are reproduced in Fig. 4. Thermal degradation of pure HDPE started at around 380°C and was completed at 470°C with complete weight loss. On the other hand, biodegraded HDPE sample showed significant weight loss in the temperature range of 20°C to 154°C. At 154°C, it had lost 16.25% of the initial weight, which may be due to the moisture content in the sample, and this reflects a moderate level of degradation of the HDPE. However, at 543°C, its weight had been reduced to 27.18°C of the initial value.

Similar is the case with pure LDPE and its biodegraded product. Thermal degradation of pure LDPE started at around 376°C and was completed with total weight loss at 466°C. On the other hand, biodegraded LDPE showed thermal degradation even at low temperature such as 20°C, and weight loss further increased with increasing temperature, and at 541°C its weight had been reduced to 28.7% of the initial weight. In this work, the weight loss at 400°C was used to compare the thermal stability of the pure HDPE and LDPE and their consortium-treated samples. It is clear from Table 4 that pure HDPE and LDPE samples showed only 2.5% and 4.5% weight loss at 400°C, whereas after treatment with consortium, the weight loss increased to 22.41% and 21.7%, respectively.

Pure HDPE showed a DTG peak temperature at 454°C at the rate of 2.0 mg/min showing two endotherms, 123°C and 455°C with heat of reactions 153 mJ/mg and 109 mJ/mg, respectively. Pure LDPE showed a DTG peak temperature at 451°C at the rate of 1.79 mg/min. It showed exotherms at 467°C with heat of reaction 53.0 mJ/mg and a pair of endotherms at 107°C and 457°C with heat of reactions 129 mJ/mg and 14.6 mJ/mg, respectively. HDPE and LDPE samples incubated with the consortium showed multiple DTG peak temperatures at significant lower rates in comparison with the pure samples (Table 3 and Fig. 4). Biodegraded samples of both HDPE and LDPE showed no exothermal peaks, but endotherms shifted to a lower temperature and heat of reactions.

Table 4. Comparative weight loss at 400°C of pure and consortium-treated HDPE and/or LDPE samples.

Treatment	Weight loss at 400°C (%)
HDPE (pure)	2.5
HDPE+consortia	22.41
LDPE (pure)	4.5
LDPE+consortia	21.7

This study suggests that microbial consortia can accelerate the rate of degradation of HDPE and LDPE under natural environments. This may be due to a direct enzymatic scission and assimilation of low-molecular-weight chains that were subsequently being produced. Moreover, the better HDPE degradation can be explained, in that there is greater cross-linking in the void volume of HDPE (higher density as compared with LDPE); this provides much more carbon content, thus resulting in enhanced sites for microbial action. Therefore, we propose to explore the indigenously developed bacterial consortium for better polyethylene waste management.

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