Environ Geochem Health
https://doi.org/10.1007/s10653-023-01590-z

ORIGINAL PAPER

Biodegradation of low-density polyethylene (LDPE) through application of indigenous strain *Alcaligenes faecalis* ISJ128

Deepa Devi · Kartikey Kumar Gupta · Harish Chandra · Kamal Kant Sharma · Kalpana Sagar · Edna Mori · Pablo Antonio Maia de Farias · Henrique Douglas Melo Coutinho · Abhay Prakash Mishra

Received: 4 April 2022 / Accepted: 18 April 2023

Abstract The resiliency of plastic products against microbial degradation in natural environment often creates devastating changes for humans, plants, and animals on the earth's surface. Biodegradation of plastics using indigenous bacteria may serve as a critical approach to overcome this resulting environmental stress. In the present work, a polyethylene degrading bacterium *Alcaligenes faecalis* strain ISJ128 (Accession No. MK968769) was isolated from partially degraded polyethylene film buried in the soil at plastic waste disposal site. The biodegradation studies were conducted by employing various methods such as hydrophobicity assessment of the strain ISJ128, measurement of viability and total protein content of bacterial biofilm attached to the polyethylene surface. The proliferation of bacterial cells on polyethylene film, as indicated by high growth response in terms of protein content (85.50 µg mL^{-1}) and viability (10^{10} CFU mL^{-1}), proposed reasonable suitability of our strain *A. faecalis* ISJ128 toward polyethylene degradation. The results of biodegradation assay revealed significant degradation (10.40%) of polyethylene film within a short period of time (i.e., 60 days), whereas no signs of degradation were seen in control PE film. *A. faecalis* strain ISJ128 also demonstrated a removal rate of 0.0018 day^{-1} along with half-life of 462 days. The scanning electron microscope (SEM) and Fourier transform infrared (FTIR) spectroscopy studies not only displayed changes on polyethylene surface but also altered level of intensity of functional groups and an increase in the carbonyl indexes justifying the degradation of polyethylene film due to bacterial activity. In addition, the secondary structure prediction (M fold software) of 16SrDNA proved the stable nature of the bacterial strain, thereby reflecting the profound scope of *A. faecalis* strain ISJ128 as a potential degrader for the eco-friendly disposal of polyethylene waste.

D. Devi · K. K. Gupta (✉) · H. Chandra · K. K. Sharma · K. Sagar
Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand 249404, India
e-mail: kartikey.gupta@gkv.ac.in

D. Devi
e-mail: dr.deepagujjar26feb@gmail.com

H. Chandra
e-mail: harish.chandra@gkv.ac.in

K. K. Sharma
e-mail: kamal.sharma@gkv.ac.in

K. Sagar
e-mail: kalpana.sagar@gkv.ac.in

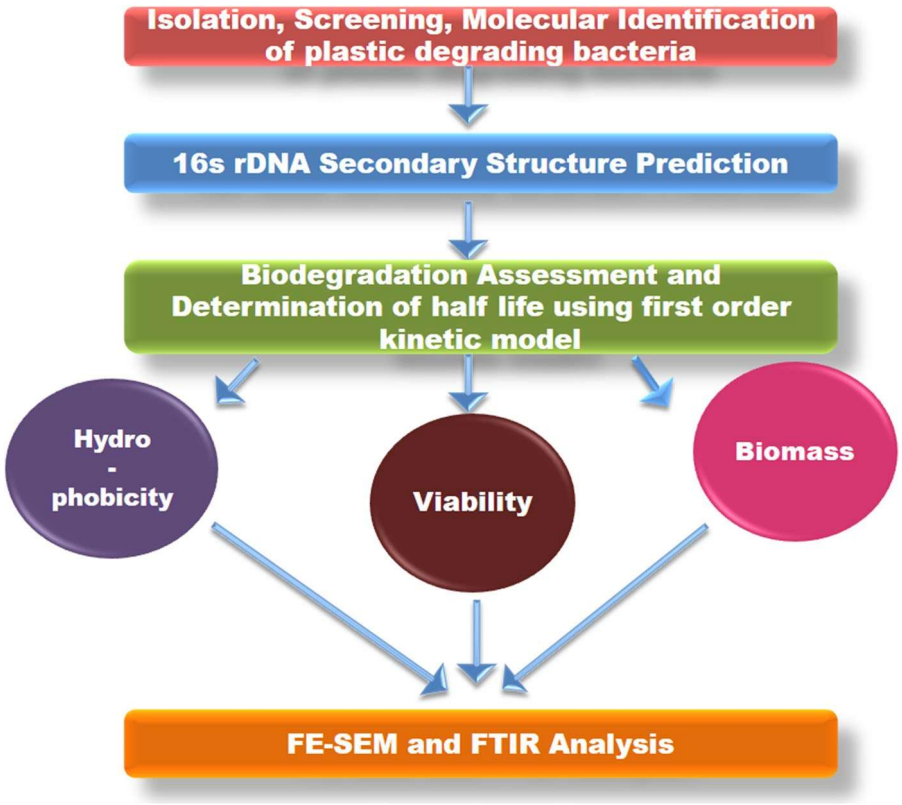
E. Mori · P. A. M. de Farias
CECAPE College, Av. Padre Cícero, 3917 - São José, Juazeiro do Norte, CE 63024-015, Brazil

H. D. M. Coutinho (✉)
Department of Chemical Biology, Regional University of Cariri - URCA, Av. Cel Antonio Luiz, 1161, Pimenta, Crato, CE 63105-000, Brazil
e-mail: hdmcoutinho@gmail.com

A. P. Mishra (✉)
Department of Pharmacology, University of Free State, Bloemfontein 9300, Free State, South Africa
e-mail: Mishra.ap@ufs.ac.za

Published online: 15 May 2023

Graphical abstract Schematic representation of methodology



Keywords Polyethylene · Biodegradation · SEM · FTIR · Biofilm

Introduction

Manufactured plastics are regarded as materials of utmost importance in modern civilization. This is attributed to their exceptional properties such as 3D structure, corrosion resistance, hydrophobicity, and so forth. Plastics, on the other hand, are indeed a vital component of our daily lives owing to their low production costs, high durability, and versatility (Ali et al., 2021; Carmen, 2021; Ru et al., 2020). Synthetic polymers are divided into two types, i.e., CC polymers and CO polymers. Polymers featuring a C spine, including polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), and polystyrene (PS), comprise about 77% of the international economy. Furthermore, CO polymers (polyethylene terephthalate—PET, polyurethane—PU, and so on) account for more than 18% of the global market. Among all, the low-density polyethylene (LDPE) has got immense applications including packaging foam, carry bag, milk packets, housewares, fluid containers, toys, and clothing, etc., due to its versatility (Carmen, 2021). Throughout 1950–2020, the entire quantity of plastics produced was found to be approximately 8.3 billion metric tonnes. The plastic production grows at a 5% annual rate (Geyer et al., 2017; Jambeck et al., 2015); however, the United Nations anticipate that plastics production will get doubled by 2035 (Velis, 2014), and reach 1800 metric tonnes by 2050 (Barra & Leonard, 2018; Gallo et al., 2018). Interestingly, around 90% of the products made with plastic are used once and then discarded. The massive usage of plastic polymers has resulted in vast amounts of plastic wastes, triggering environmental concern (Barnes et al., 2009; Cózar Cabañas et al., 2014; Galgani et al.,

2019; Jambeck et al., 2015; Morét-Ferguson et al., 2010; Rochman et al., 2013; Wagner et al., 2014). According to Geyer et al. (2017), around 12,000 million tons of plastic waste will be accumulating in the environment till 2050. Unfortunately, according to recent figures, 76% of all plastic output is eventually discarded. Out of which, 9% is recycled, 12% is incinerated, and 79% being subjected to landfill or may end up in the marine and terrestrial habitats (Garcia and Robertson, 2017; Geyer et al., 2017). These plastic wastes eventually find their way to water bodies and landfill sites, where they remain for decades, polluting the environment and posing a serious threat to most life forms, including all types of terrestrial ecosystems such as deserts, forests, grasslands, and polar regions (Gregory, 2009; Zylstra, 2013). The PE waste buried in soil may affect the drainage system, disturb soil fauna, and decrease the soil quality leading to a declined agricultural yield (Ali et al., 2021). Plastic pollution also has a negative impact on the aquatic environment, as evidenced by a decrease in the number of marine species (Browne et al., 2011). After disposal, the degradation of plastics such as LDPE in the natural environment is extremely difficult, which directly pollutes the environment and disturbs the ecosystem which may raise the concern about sustainable development (Kumar et al., 2021). Concept of sustainable development was discussed in 1972 as part of the United Nations Conference (Stockholm) (Mata-Lima et al., 2016). The environmental degradation has been listed as a serious issue according to the agenda 2030 for sustainable development as discussed in 2015 at the Rio20, the conference for sustainable development (de Sousa et al., 2021).

Therefore, elimination of these hazardous substances from the environment has now become a prerequisite requirement of the present era. However, a number of conventional methods, i.e., landfilling, incineration, chemical treatment, and thermal degradation used in the management of polyethylene waste, are already known to have hazardous effects on the environment including living organisms (Yang et al., 2011). For instance, direct incineration of polyethylene waste produces vapors composed of many toxic carcinogenic compounds like ketones, acrolein, along with greenhouse gases like methane exerting serious health and environmental problems for humans (Briassoulis, 2004; Patel et al., 2021). The increasing levels of LDPE waste, decrease landfill capacity. The serious disadvantages associated with conventional methods of waste management have caused an urgency to carry out remedial measures on polyethylene waste. An efficient and eco-friendly approach suitable for plastic waste management is still missing. In this concern, several reports are focusing on the possibility of microbial degradation of plastic waste (Sarkhel et al., 2020; Zhang et al., 2020). Microorganisms play an important part in the biological decomposition of plastics materials (Shah et al., 2008). However, a major obstacle to biodegradation of LDPE is due to its resistance toward biological attack resulting from its three-dimensional structure, high molecular weight, hydrophobic nature, and lack of functional groups recognizable by microbial enzymatic systems (Hamid, 2000; Harshvardhan & Jha, 2013).

Many studies have been conducted on the investigation of biodegradation of PE in natural environmental conditions including seawater, soil, compost, and sludge (Albertsson et al., 1987; Hadad et al., 2005; Karlsson et al., 1988; Kumar et al., 2007; Lee et al., 1991; Nanda & Sahu, 2010; Sudhakar et al., 2008). In biodegradation studies, several bacterial and fungal isolates have been shown to be capable of degrading polyethylene polymer; however, breakdown rates may vary and are often modest even after prolonged treatment period (Restrepo-Flórez et al., 2014). Polyethylene biodegradation is aided by a variety of microbes that includes *Rhodococcus* (Sivan et al., 2006), *Bacillus* (Novotný et al., 2018; Sudhakar et al., 2008), *Brevibacillus* (Hadad et al., 2005), *Kocuria* (Harshvardhan & Jha, 2013).), *Pseudomonas* (Gupta & Devi, 2020; Kyaw et al., 2012), *Acinetobacter* (Pramila & Ramesh, 2015), *Micrococcus* (Gupta et al., 2022). Although the exploration of microorganisms toward the polyethylene degradation is well studied, the quest for novel microorganisms with enhanced biodegradation ability is the need of the present period (Reddy, 2008; Tribedi & Sil, 2013).

Alcaligenes faecalis is a Gram-negative, obligate aerobic, oxidase-positive, catalase-positive, non-fermenting bacteria. It is often found in soil, water, and in hospital settings and has been shown to be important in the biodegradation of ochratoxin A (Zhang et al., 2017), the breakdown of the organic pesticide endosulfan (Kong et al., 2013), and the degradation of chrysene and diesel oil (Igwo-Ezikpe et al., 2009). Furthermore, it is a favorable bacterium for

polyethylene breakdown (Nag et al., 2021). It has been shown that *A. faecalis* secretes extracellular enzymes capable of degrading polyethylene through surface degradation, such as lipase, CMCase, xylanase, and protease (Nag et al., 2021). Moreover, the extracellular polymers released by this microbe function as surfactants, promoting the interchange of hydrophilic and hydrophobic phases. Such encounter favors the growth of microbes on to the polyethylene surfaces. However, studies on biodegradation of polyethylene, particularly LDPE, by *Alcaligenes* bacterium are scarce. In the current paper, biodegradation of LDPE has been investigated, utilizing an indigenous bacterial strain *A. faecalis* ISJ128 isolated from waste disposal sites.

Materials and methods

Screening of bacterial isolates for polyethylene degradation

Pre-treatment of PE sheets

Transparent low-density polyethylene (LDPE) sheets generally used for packaging were purchased from VSPN packaging industries, Bhagwanpur, Haridwar (Uttarakhand), India. LDPE sheets were cut into minor sections and sterilized with 70% ethanol, followed by heating with xylene at 60 °C for 5–15 min until the polyethylene dissolved completely. The resulting slurry was cooled by immersing the beaker in an ice bath. The solvent was extracted from the slurry using sterilized muslin cloth. The residue was further washed with ethanol for 2–3 times to remove the residual xylene. The resulting polyethylene powder was kept as such to evaporate remaining ethanol and then dried overnight in hot air oven at 60 °C. Finally, the polyethylene powder was stored at room temperature for further experimental analysis (Azeko et al., 2015; Bhatia et al., 2014).

Isolation of LDPE degrading bacterial strains

Partially degraded polyethylene films were collected from waste disposal site of Rishikesh, Uttarakhand (30° 07′ 35.76″ N and 78° 16′ 34.86″ E), in order to isolate polyethylene degrading bacterial strain with all necessary precautions (Fig. 1). The sample was collected randomly from the superficial layer of soil (5–15 cm) in depth, using pre-sterilized spatula in sterile zip-lock bags (HiMedia) by using aseptic techniques, sealed properly, transported to the laboratory, and analyzed within 24 h after collection (Zaki et al., 2012). Bacterial strains were isolated on nutrient agar by employing serial dilution and spread plate technique. The plates were incubated at 37 °C for 24–48 h. Colonies with varying morphological traits were chosen and sub-cultured on nutrient agar for further investigation (Pepper et al., 1995).

Selection of PE degrading bacterial strains using clear zone assay

The bacterial isolates obtained from waste disposal site were screened for polyethylene degradation on minimal salt medium (MSM) containing dipotassium hydrogen phosphate (0.1 g L^{-1}), potassium dihydrogen phosphate (3.0 g L^{-1}), sodium chloride (5.0 g L^{-1}), ammonium chloride (2.0 g L^{-1}), magnesium sulfate (0.2 g L^{-1}), calcium chloride (0.1 g L^{-1}), KCl (0.15 g L^{-1}), and agar powder (15 g L^{-1}) incorporated with 1 g L^{-1} of fine LDPE powder as the sole carbon source (added to the medium after sterilization to avoid deformation (Russell et al., 2011). The bacterial strains were streaked on mineral salt medium containing fine LDPE powder as only carbon source; then, the plates were incubated at 30–35 °C for

Fig. 1 Photograph of sampling site from where partially degraded polyethylene was collected for the isolation of bacteria

14–28 days (Skariyachan et al., 2015). After completion of incubation period, the inoculated MSM plates were treated with Coomassie brilliant blue R-250 (0.1%) for 20 min. The solution was then poured off as well as the plates were again flooded for 20 min with a de-stain solution containing 40% (v/v) methanol and 10% (v/v) acetic acid. After this, isolates showing zone of clearance against a blue background were considered as positive for the test and selected as the utilizer of polyethylene (Howard and Hilliard 1999). The isolates showing positive results in clear zone test were repeatedly sub-cultured on nutrient agar medium to obtain pure culture of bacterial isolates and stored at 4 °C.

Identification of bacterial strain ISJ 128

Morphological and biochemical characterization

The bacterial strains were differentiated through colony morphology, microscopic examination, and biochemical test. Morphological characterization of the clear zone-positive isolate was done by Gram's staining method (Beveridge, 2001). Further characterization was carried out using the criteria outlined in Bergeys's manual of determinative bacteriology (Holt et al., 1994). Standard physiological and biochemical properties, e.g., oxidase and catalase production, lipid, starch and casein hydrolysis, gelatin liquefaction, nitrate reduction, and acid production from carbohydrates, were determined by conventional methods (Chandra et al., 2016; Shah et al., 2014).

Molecular characterization

The genomic DNA of bacterial strain ISJ128 was isolated using the method described by Ganesan et al., 2017. The 16S rRNA genes were amplified by polymerase chain reaction with dNTP, Buffer, Taq polymerase and by using universal primer sequence AGA GTTTGATCMTGGCTCAG and CGGTTACCTTGT TACGACTT. Thirty-five cycles of PCR were performed, and the finally obtained product was stored at 4 °C. After that the amplified product run on 1% agarose gel at 80 V for 60 min and the gel was visualized under UV light. Automated sequencing was carried out according to the Sanger di-deoxy chain-termination method. The obtained DNA sequences were compared with the reference species of bacteria, contained in genomic database, using the BLAST tool in NCBI platform (http://www.ncbi.nlm.nih.gov/BLAST), and similarity between the strains was matched. The DNA sequences were aligned, and MEGA X software was used to create a phylogenetic tree using phylogeny bootstrapping and the neighbor-joining method. The 16S rRNA sequence was deposited in the GenBank, NCBI database.

16 s rDNA folding prediction and restriction sites analysis

The secondary structure of the 16S rDNA was predicted by Mfold software (http://unafold.rna.albany.edu/) to analyze the structural stability in terms of Gibbs free energy (Zuker, 2003). The sequences of bacterial strain ISJ128 were submitted to the Mfold web server, and the parameters such as exterior loop type, bulge loop size, base numbering frequency, structure draw mode, structure annotation, and structure rotation angle were fixed. The folding of 16 s rDNA was anticipated to occur at 37 °C in 1 M NaCl ionic environment devoid of divalent ions, and restriction sites were identified using NEB cutter (v 2.0) (nc2.neb.com/nebcutter2/) (Gupta & Rana, 2021).

Biodegradation assessment of PE Films using bacterial strain ISJ128

In the present work all assays for evaluating the bacterial biodegradation were performed employing Bushnell Haas Broth (BHM) medium, containing the following elements in distilled water (g L^{-1}): NH_4NO_3 1.0; K_2HPO_4 1.0; $MgSO_4$, 0.2; KCl 0.15; $CaCl_2$ 0.1; and each of the following microelements: 1.0 mg, $FeSO_4$, 1.0 mg, $ZnSO_4$, and 1.0 mg $MnSO_4$. 0.1 g of LDPE samples (3×3 cm) was weighed and disinfected with 70% ethanol for 30 min followed by drying overnight at 60 °C (Das & Kumar, 2015). The samples were then air-dried for 15 min in a laminar airflow chamber and added to conical flasks containing 100 ml of Bushnell Hass Broth. After that LDPE-supplemented medium was inoculated with 5 ml culture of (24 h old) bacterial strain ISJ128 followed by incubation for 60 days on a rotary shaker at 180 rpm and 35 °C. Un-inoculated BHM along with polyethylene film as a substrate used as control. The polyethylene films were removed from the medium at every

20 days of interval to measure weight loss in residual films. The bacterial biofilm was removed from polymer surface by soaking in 2% (v/v) sodium dodecyl sulfate solution (SDS) for 4 h (Awasthi et al., 2017). Following that, the PE films were washed with distilled water before being washed with 70% ethanol to ensure most effective biofilm removal. The washed polymer pieces were placed on a filter paper and dried overnight at room temperature before weighing.

$$\%\text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Assessment of hydrophobicity

The hydrophobicity of the bacterial cell surface was determined using the bacterial adhesion to hydrocarbon (BATH) test (Rosenberg et al., 1980). In this assay, bacterial isolates were cultured in the medium until they reached the mid-logarithmic phase, then centrifuged (5000 rpm), and washed repeatedly with phosphate urea magnesium (PUM) buffer containing following compositions (per liter): 17 g K_2HPO_4, 7.26 g KH_2PO_4, 1.8 g urea, and 0.2 g $MgSO_4.7H_2O$. The washed cells were resuspended in PUM buffer to an optical density at 600 nm (OD_{600}) value of 1.0–1.2. Aliquots (1.2 ml each) of this suspension were transferred to a set of test tubes, to which increasing volumes (range 0–0.2 ml) of hexadecane were added. To facilitate phase separation, the test tubes were shaken for 10 min and then allowed to stand for 2 min. The OD_{600} of the aqueous phase was measured with cell-free buffer serving as reference.

Viability and biomass of strain ISJ 128

The treated LDPE films were removed from the synthetic medium using sterile forceps and gently washed with ultrapure water to remove loosely adherent bacterial cell. The bacterial biofilm on LDPE surface was removed by water bath sonication in 1 mL of 0.85% saline solution. After sonication, the resulting saline solution thus obtained was used to determine viability and biomass of bacterial biofilm adhered on PE surface. The viability was monitored (in term of CFU mL^{-1}) on every 20^{th} day using serial dilution and spread plating technique, while biomass estimated in the form of protein concentration determined spectrophotometrically at 595 nm using Bradford assay (Arkatkar et al., 2010).

Field emission scanning electron microscopy (FE-SEM) of PE film

The LDPE films were recovered from the medium (after 60 days), washed in phosphate buffer (0.01 M) for 2 min, and then examined using scanning electron microscopy (SEM) to confirm biofilm formation by the strain ISJ128. To visualize topographical changes on surface of LDPE, the polymer was washed with 2% (v/v) sodium dodecyl sulfate (SDS) and ultrapure water for 5–8 min to eliminate bacterial biomass followed by treatment in 4% glutaraldehyde at 4 °C for 90 min and dehydrated with ethyl alcohol (50%) for 25 min followed by overnight incubation with 70% ethanol, dried, sputter coated with gold for 40 s, and scanned at 20 kV and 20000X magnification under scanning electron microscope (Quanta 200 FEG; Thermo Fisher Scientific, Waltham, MA) (Kyaw et al., 2012).

Fourier transform infrared (FTIR) spectroscopy

Modification in the structural and functional groups of polyethylene films was analyzed by Fourier transform infrared (FTIR) (Perkin Elmer, Spectrum EX) spectra in the frequency range of 4000–400 cm^{-1}. Furthermore, the absorbance of carbonyl index (the ester carbonyl bond, keto carbonyl bond, terminal double bond (vinyl), and the internal double bond) was evaluated using the following formula: keto carbonyl bond index (KCBI) = I1715/I1465; ester carbonyl bond index (ECBI) = I1740/I1465; vinyl bond index (VBI) = I1650/I1465; and internal double bond index (IDBI) = I908/I1465. Carbonyl index was used to measure that the degree of biodegradation has its value depending on the degradation.

Results and discussion

Isolation and screening of PE degrading bacteria

In the present study, eight bacterial isolates from the soil adhered with partially degraded polyethylene

film were evaluated for their ability to degrade LDPE. The bacterial isolates were streaked on minimal salt agar plate and incubated for 28 days at 35 °C. After staining with Coomassie brilliant blue, one of these isolate ISJ128 produced clear zone around its colony, demonstrating the development of exo-enzymes that degraded and utilized LDPE as sole carbon source present in minimal media. It was apparent from the literature survey that the clear zone assay is very straightforward and advantageous technique for screening polyethylene degrading bacteria since the development of clear zone around bacterial colony obviously demonstrates the discharge of exo-enzymes, which inevitably brings about polymer solubilization (Augusta et al., 1993). It was confirmed in the preliminary study that strain ISJ128 was capable of growing on synthetic minimal salt media by producing clear zone around the colony; in this manner, strain was additionally chosen for performing biodegradation studies on LDPE films (Table 1).

Phenotypical and molecular characterization of ISJ128

Characteristic analysis of ISJ128 revealed isolate ISJ128 to be a Gram-negative, non-spore forming bacteria. The morphological and biochemical characteristics are mentioned in detail in Table 2. The sequence obtained was subjected to basic local alignment search tool (BLAST) (http://blast.ncbi.ntm.nih.gov) and deposited in NCBI with Accession No. MK968769. The phylogenetic analysis of the 16S rRNA sequence revealed that the strain ISJ128 belonged to genus *Alcaligenes* and possessed 100% similarity with several strains of *A. faecalis,* but the closest one was *A. faecalis* strain BBRC13111 (BBJQ0100002499) based on maximum score (Fig. 2). The nucleotide sequence reported here can be obtained from NCBI nucleotide sequence database under accession number MK968769.

Table2 Morphological and biochemical properties of ISJ128

Characteristics	ISJ128
Colony morphology	
Color	Off white
Surface	shiny
Margin	entire
Cell morphology	
Gram staining	−
Spore formation	−
Shape	Rod
Biochemical tests	
Casein hydrolysis	+
Starch hydrolysis	−
Gelatin liquefaction	−
Oxidase	+
Catalase	
Citrate utilization	−
Voges-Proskauer	+
Methyl red	−
Carbohydrate fermentation	
Fructose	+
Sucrose	−
Mannose	−
Raffinose	−
Lactose	−
Sorbitol	+

Prediction of 16S rRNA secondary structure and restriction sites analysis

The concept of analyzing free energy linked with the folding of 16S rRNA gene sequences from bacterial isolates could provide crucial information for making concurrent prediction on gene stabilities. The 16S rRNA folding was anticipated to explain the evolutionary stability of ISJ128 gene sequences (Fig. 3). The free Gibb's energy of 16S rRNA in its folded form was observed to be -545.90 kcal/mol for *A. faecalis* ISJ128 (Fig. 3a), respectively. This study suggested the minimal energy level of

Table 1 Isolation and screening of bacterial isolates showing positive results for preliminary screening

Sample code	Sampling site	Coordinates	Isolated bacterial strains	Positive for clear zone
SAN15	Ganga vatika, Rishikesh	30° 07′ 35.76″ N and 78° 16′ 34.86″ E	ISJ126, ISJ127, ISJ128, ISJ129, ISJ130, ISJ131, ISJ132, ISJ133	ISJ128

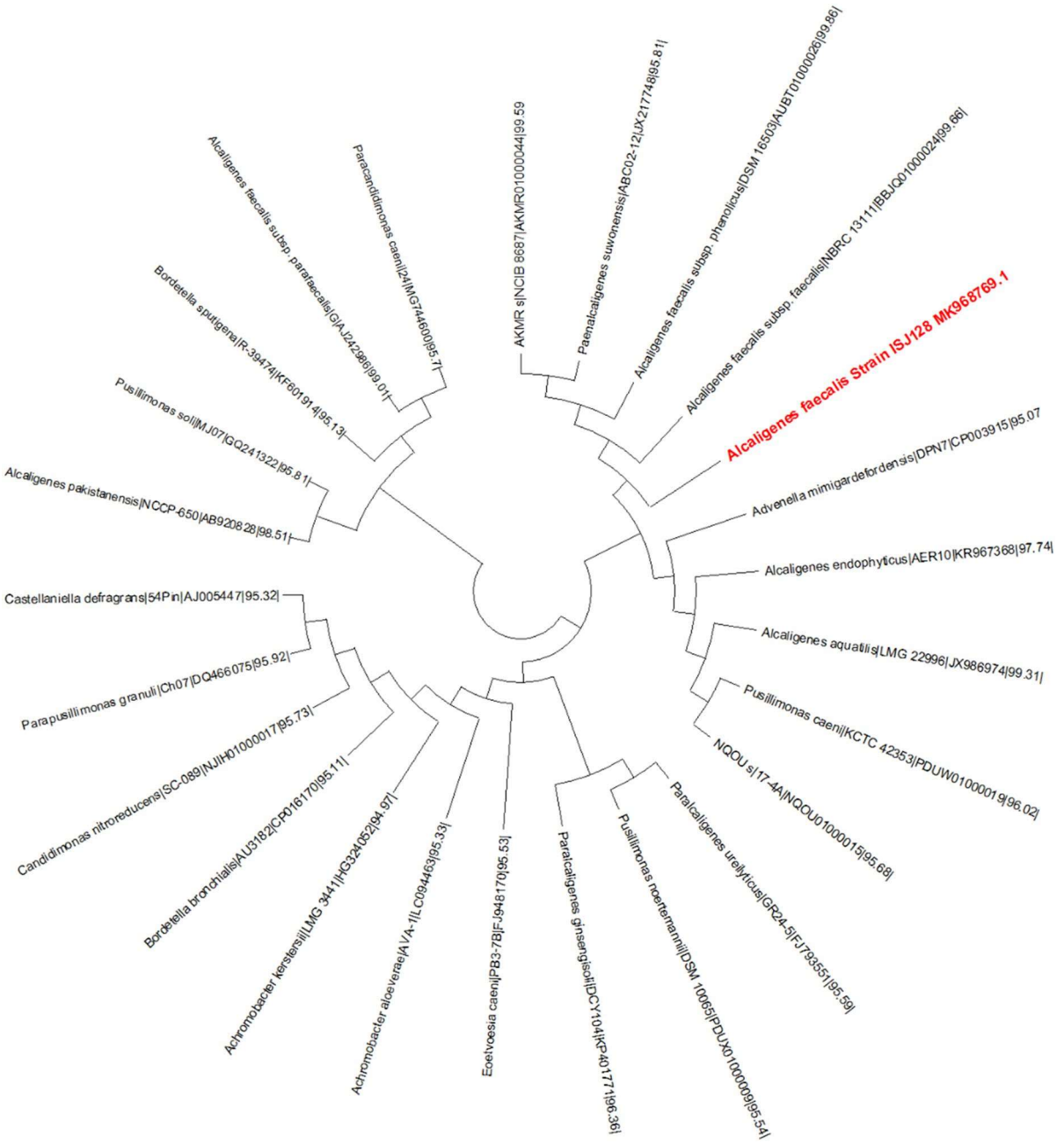


Fig. 2 Phylogenetic relationship of *A. faecalis* ISJ128 based on 16S rRNA gene nucleotide sequences. Numbers at the nodes indicate bootstrap values from the neighbor-joining analysis of resampled data sets

16S rRNA sequences, specifying high folding stability of nucleotides in the organisms. The restriction analysis of 16S rRNA sequence of *A. faecalis* strain ISJ128 showed 54% and 46% GCAT content, respectively (Fig. 3b).

Biodegradation assessment of PE films using bacterial strain ISJ128

Weight reduction of polyethylene is a simple and efficient method of quantifying biodegradation. The

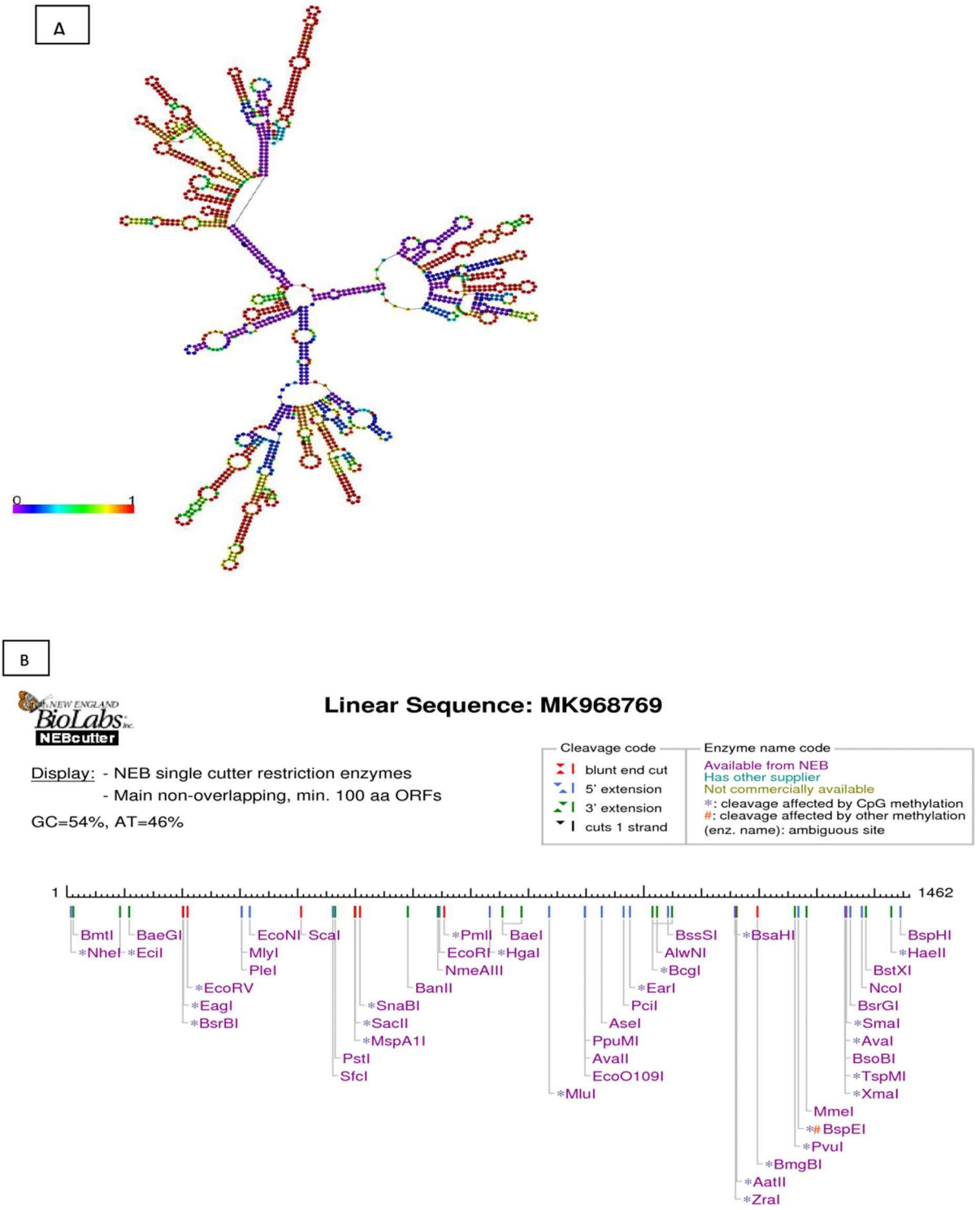


Fig. 3 Predicted secondary structure of 16S rRNA isolated from bacterial strain *A. faecalis* ISJ128 **(A)**. Restriction sites on the 16S rRNA sequence of *A. faecalis* ISJ128 **(B)**

adaptive response of bacterial cell on PE surface is the initial requirement for biodegradation (Gupta et al., 2016). The experiments were conducted in triplicates, and reduction in the weight of treated LDPE polymer was recorded after 20, 40, and 60 days during incubation period (Fig. 4). The signs of significant weight loss (10.4%) were detected in LDPE after treatment with *A. faecalis* ISJ128 after completion of incubation period (60 Days), whereas no signs of degradation were observed in control LDPE films. Statistical analysis results indicated that the reduction in the weight of LDPE with incubation period was significant ($p < 0.05$). The results clearly demonstrated that bacterial isolate ISJ128 exhibited significantly higher weight loss after completion of incubation period. Similar findings were reported by Harshvardhan and Jha (2013) for *K. palustris, B. pumilus,* and *B. subtilis* responsible for the reduction in the weight of LDPE by $1 \pm 0.033\%$, $1.5 \pm 0.038\%$, and $1.75 \pm 0.06\%$, respectively. On the other hand, in another study, Auta et al. (2017) recorded 1.6% weight loss for polyethylene by *Bacillus cereus*. Comparable findings have been accounted for by different researchers (Kyaw et al., 2012; Farzi et al., 2019; Gupta & Devi, 2020; Gupta et al., 2022). Moreover, removal rate constant and half-life of residual LDPE were also calculated using first-order kinetic model where *A. faecalis* strain ISJ128 exhibited a shorter half-life of 462 days along with an expected removal rate constant of 0.0018 day^{-1}.

Assessment of hydrophobicity

The propensity of bacteria to utilize any substrate as a carbon and energy source is largely determined by their adhesion or growth to that substrate (Rosenberg et al., 1980). In the present study, a BATH assay was performed to investigate the interaction between the bacterial isolate and polyethylene in Fig. 5. In carbon-starved conditions, the bacterial surface turns out to be more hydrophobic and adhesive than non-starved condition (Kavitha & Bhuvaneswari, 2021). The development of an efficient biofilm is the consequence of broad bacterial colonization on LDPE surface. The BATH test findings demonstrate that ISJ128 cells had 33.5% hydrophobicity in 0.2 mL

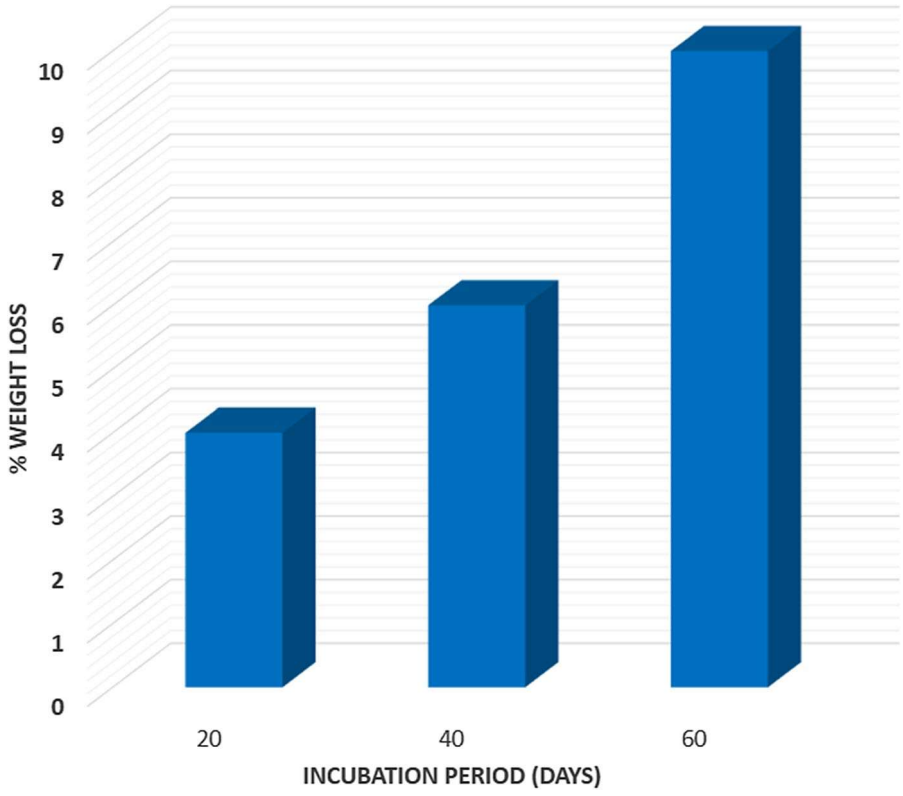


Fig. 4 Percentage biodegradation of polyethylene film treated with bacterial strain ISJ128. Value represents the average of three independent experiments

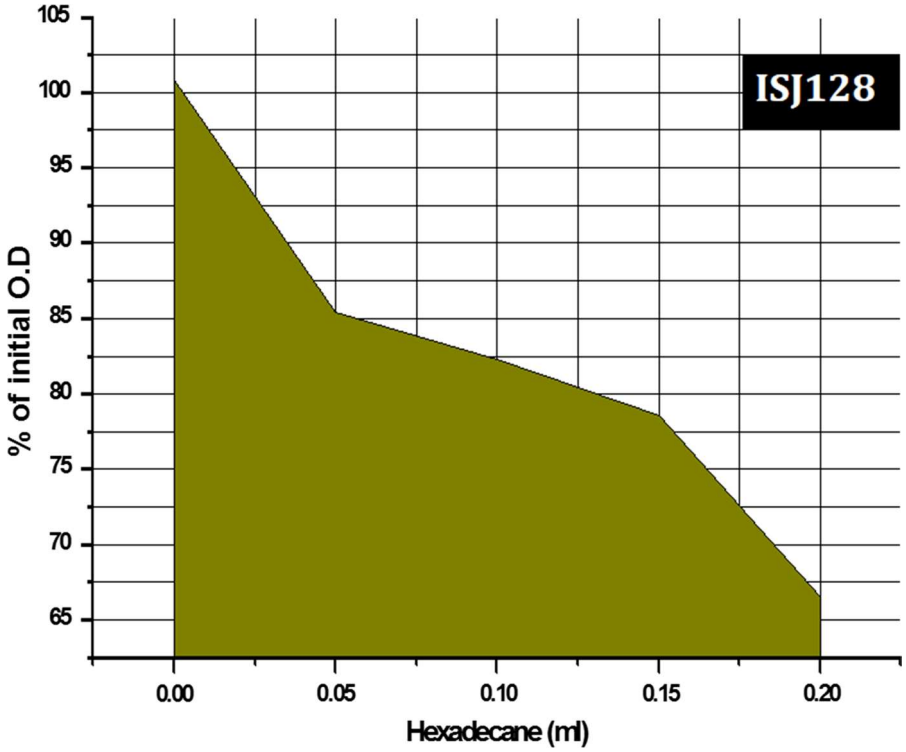


Fig. 5 Waterfall graph showing % decrease in hydrophobicity of bacterial isolate ISJ128 upon increasing concentration of hexadecane (ml). O.D (optical density) was determined spectrophotometrically at 600 nm by BATH test

of hexadecane (Fig. 5). The relocation of bacterial cells from aqueous phase to hexadecane is conceivably connected to a decrease in turbidity of fluid progressively work featuring the capacity of our strain ISJ128 in adhering to polymer surface. In the view of this study, strain ISJ128 produces a significant measure of biofilm on the LDPE surface which was later confirmed by FE-SEM investigation. It is clear from the experimental values that ISJ128 displayed a significant reduction in turbidity of aqueous phase even at a low concentration (0.2 ml) of hexadecane, indicating its strong adherence to hydrophobic surface. Formation of biofilm on hydrophilic or hydrophobic surface is influenced by a number of factors such as properties of substrate as well the force that attracts the bacteria toward polymer surface. As per literature survey, Harshvardhan and Jha (2013) observed a 10% and 24% decrease in turbidity of *Bacillus subtilis* and *Bacillus pumilus,* respectively. In another research, Orr et al. (2004) documented a significant reduction of cell surface hydrophobicity (20%) for *Rhodococcus ruber* strain. In the view of this study, we state a decrease in turbidity of the aqueous phase of ISJ128, following addition with hexadecane. Because of the hydrophobic nature of polyethylene, an organism with a greater cell surface hydrophobicity is predicted to utilize the polymer more efficiently since hydrophobicity facilitates the interaction of bacteria with polyethylene (Orr et al., 2004). Our findings support this activity since they displayed excellent adherence to hydrocarbons.

Viability and biomass of strain ISJ 128

Low-density polyethylene surfaces were rapidly colonized by the isolate during first 20 days of incubation. A persistent rise in ISJ128 biofilm density was noted throughout the experiment right up to the last day of the incubation period (Fig. 6a). Maximum biofilm density, i.e., 1.24×10^{10} CFU $mL^{-1,}$ was noted after 60 days of incubation. During the incubation period, the density of planktonic cells of *A. faecalis* ISJ128 was also found to be increased from 1.0×10^{10} (20th day) to 1.3×10^{10} (60th day). The root cause of this increase might be those bacterial cells that disengaged from the active biofilm over polymer surface. Our finding suggests that increased bacterial cell hydrophobicity facilitates effective biofilm formation by ISJ128 on polyethylene film. It has been found in published research that microorganisms frequently

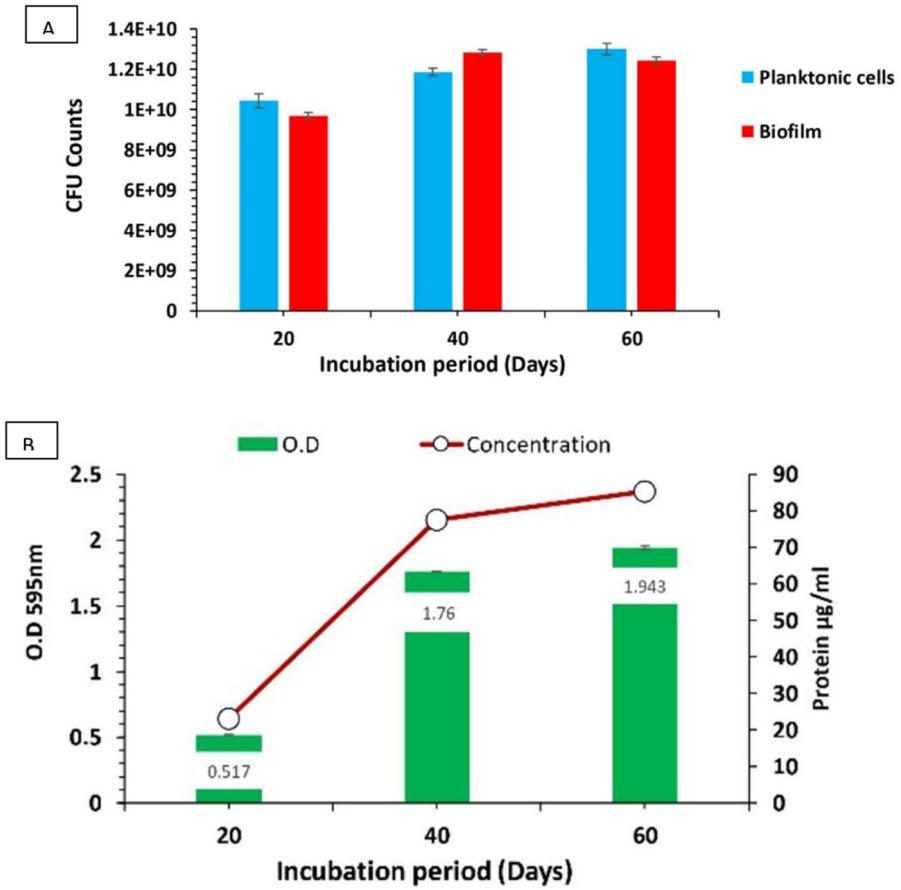


Fig. 6 Viable cell counts (CFU mL^{-1}) of bacterial strain ISJ128 values represent the mean of three independent experiments. Bars indicated standard error mean (±SEM). Statistical significance was calculated by one-way ANOVA at $p < 0.05$ significance level (**A**). Protein contents of bacterial cultures in Bushnell Hass Broth medium supplemented with polyethylene film values representing the mean of three independent experiments. Bars indicated standard error mean (±SEM). Statistical significance was calculated by one-way ANOVA at $p < 0.05$ significance level (**B**)

form biofilm on polymer surfaces, resulting in better remediation mainly due to higher microbial biomass, i.e., CFU mL^{-1}, resulting in enhanced adaptability of bacterial cells on polymer surface (Bhatia et al., 2014; Sudhakar et al., 2008). The viability of bacterial cell on the surface of LDPE was monitored after every 20 days during 60 days of incubation period. The data clearly showed that bacterial strain ISJ128 initiated the development of biofilm within the first 20 days (Fig. 6a). A rise in the growth rate of bacteria during first 20 days of treatment does not essentially suggest a high response and enhanced efficiency of the isolates but rather a period that permits microorganisms to interact favorably with the polymer surface and facilitating rapid metabolism (Gupta et al., 2022). Thus, it appears that in the presence of LDPE films our isolate, namely *A. faecalis* ISJ128, displayed a remarkable adaptability in carbon-starved conditions and developed a thick biofilm matrix on polymer surface.

The quantity of total protein content (µg mL^{-1}) was estimated as O.D (Fig. 6b). The total protein content was used to determine the progression of biofilm formation on PE surface with time. Results depicted in Fig. 6b exhibited a pattern similar to that of the viability of the biofilm measured in the form of CFU/cm^2. In this work, we observed the highest level (85.3 µg mL^{-1}) protein content of *A. faecalis* ISJ128 after 60 days of incubation, indicating a significant amount of bacterial population on PE surface as the incubation progressed. This increase in protein content during incubation reflects an increase in surface-attached biomass, which may suggest that biomass on LDPE films is proliferating continuously. The results are similar to those of Duddu et al. (2015) who reported 70 µg mL^{-1} total protein content of surface-attached biomass of *Streptomyces* sp. Arkatker et al. (2010) reported biomass for *Pseudomonas azotoformans*, *Bacillus flexus,* and *Bacillus subtilis* ranging from 190 to 244 µg mL^{-1} on UV-treated

polypropylene surface. However, protein content for thermally treated polypropylene for the same bacteria isolates ranged from 44 to 48 µg mL^{-1} after an incubation of 12 months. On the other hand, Orr et al. (2004) demonstrated 4.1 µg mL^{-1} protein concentration of *R. ruber* for UV pretreated polyethylene after 30 days.

Surface analysis of degraded PE

Formation of biofilm and deformation of surface micromorphology of polyethylene films were visualized with scanning electron microscopy (SEM). As seen in Fig. 7B and C, a dense biofilm was formed on polyethylene surface after 60 days of incubation. The presence of fully covered biofilm of ISJ128 on polyethylene surface demonstrates its excellent adhesion capabilities to polyethylene. The analysis of control and bacterial treated polyethylene films indicates changes in the polymer surface. The untreated sample maintained intact surface integrity without any significant changes (Fig. 7A), whereas polyethylene films treated with ISJ128 exhibited degrading signs on PE surface in the form of surface bioerosion such as cracks, pits, voids, and folding on its surface (Fig. 7D). Aravinthan et al. (2016) observed consistent results after exposing polypropylene to *B. flexus* and *B. subtilis* for one year. This could be due to an exo-enzyme produced by bacteria in response to nutritional stress. Surface bioerosion is the primary cause of mass loss from the surface. Many researchers have documented the similar morphological changes in LDPE breakdown by microorganisms (Auta et al., 2017; Farzi et al., 2019; Kyaw et al., 2012).

Fourier transform infrared spectroscopy

FTIR is a spectroscopic analytical technique used to detect chemical groups transformation. FTIR spectra

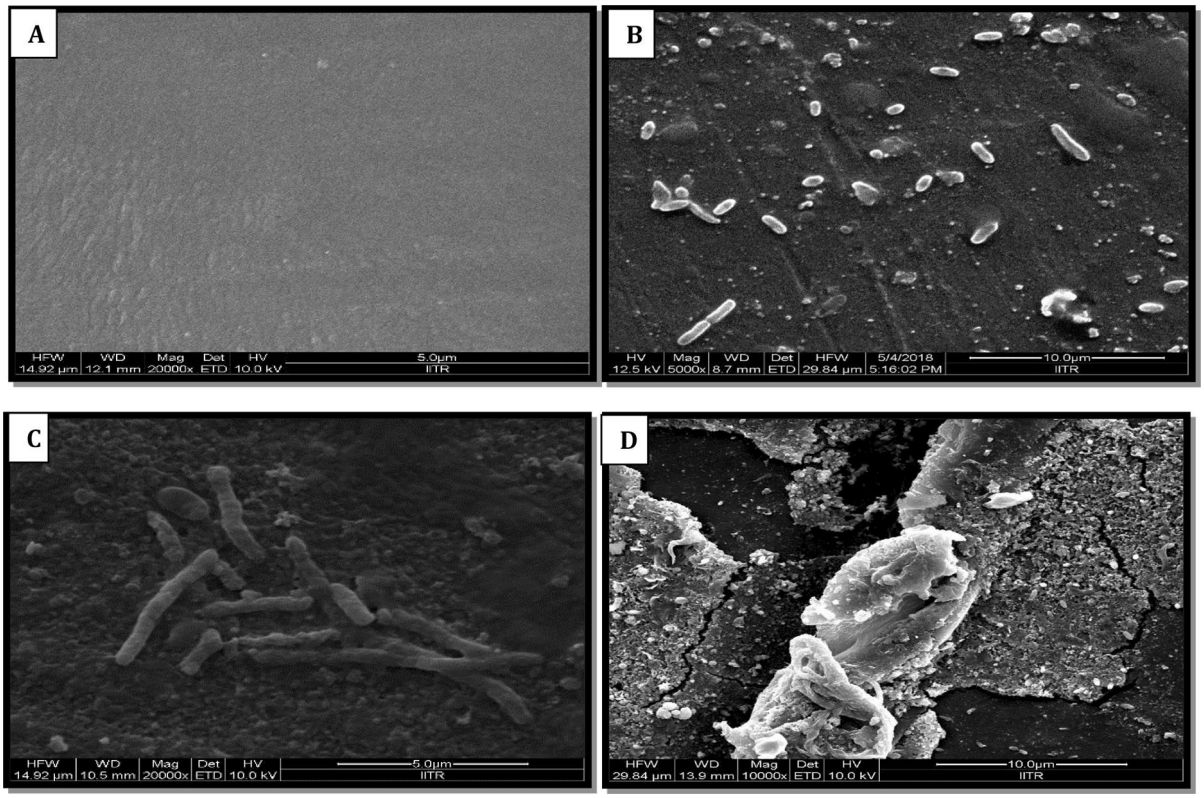
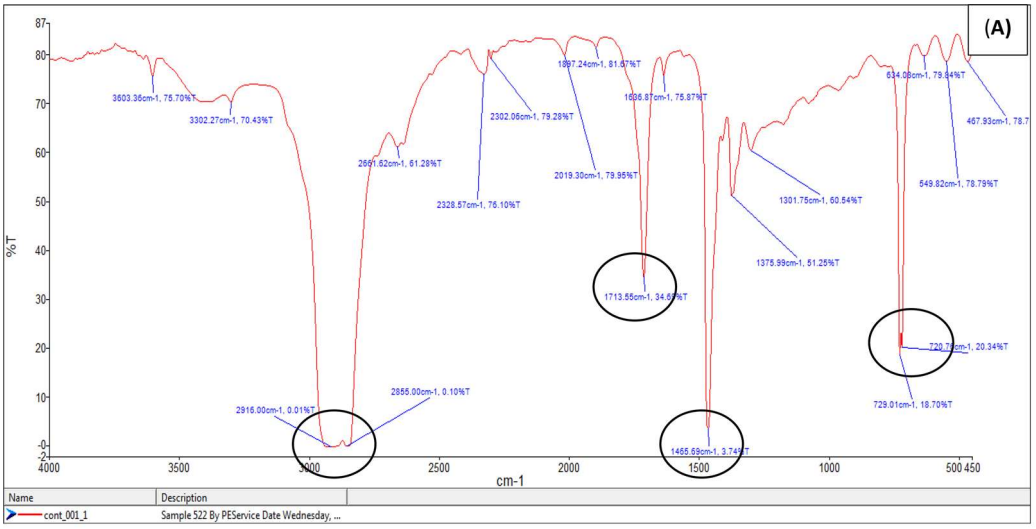
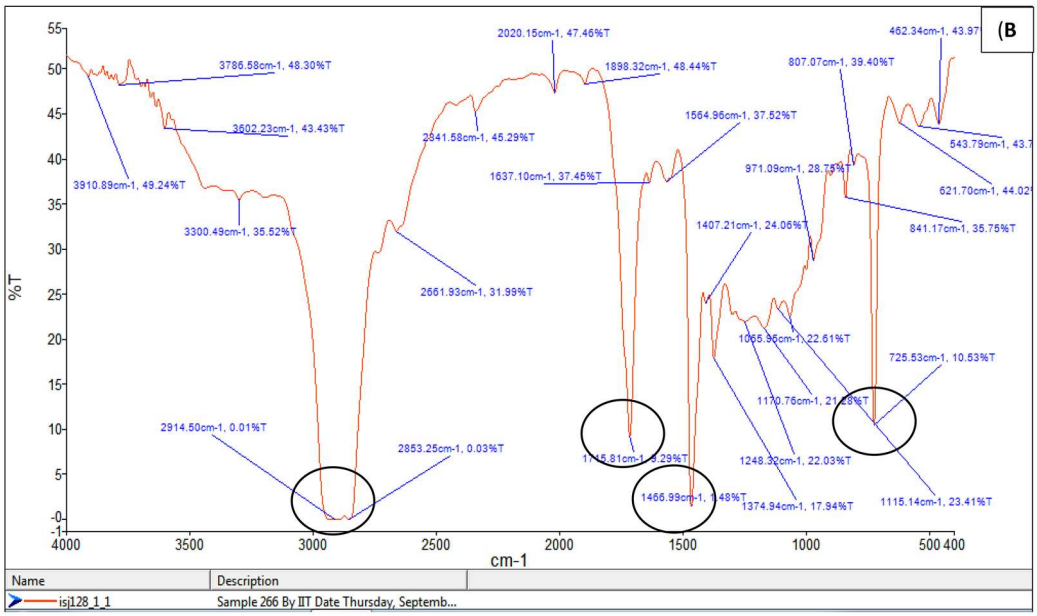
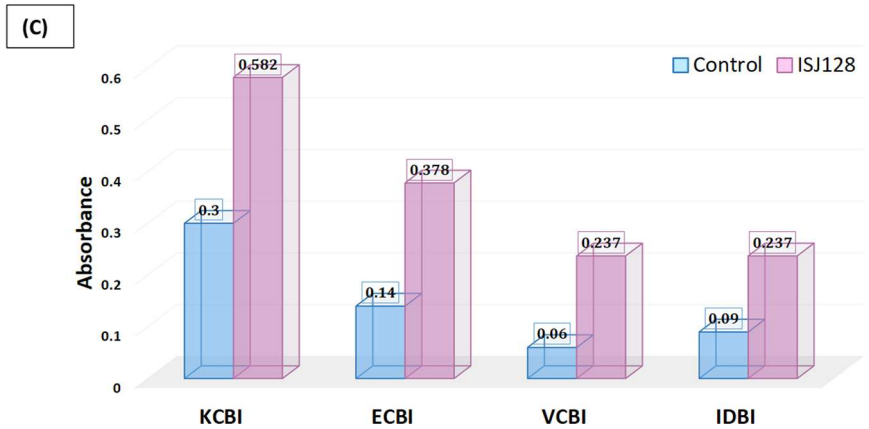


Fig. 7 Scanning electron microscope (SEM) photographs of colonization and biofilm formation by *A. faecalis* strain ISJ128 on PE, blank (untreated) (**A**). A mature biofilm after 60 days (**B**). Three-dimensional structures of *A. faecalis* ISJ128 (**C**). Formation of pits and cracks on the polyethylene surface after 60 days of incubation (**D**)


(A)
%T
cm-1
3603.36cm-1, 75.70%T
3302.27cm-1, 70.43%T
2916.00cm-1, 0.01%T
2855.00cm-1, 0.10%T
2691.62cm-1, 61.28%T
2328.57cm-1, 76.10%T
2302.06cm-1, 79.28%T
2019.30cm-1, 79.95%T
1997.24cm-1, 81.67%T
1636.87cm-1, 75.87%T
1713.55cm-1, 34.67%T
1465.59cm-1, 3.74%T
1375.99cm-1, 51.25%T
1301.75cm-1, 60.54%T
634.08cm-1, 79.84%T
549.82cm-1, 78.79%T
729.01cm-1, 18.70%T
Name
Description
cont_001_1
Sample 522 By PEService Date Wednesday, ...


(B
%T
cm-1
3786.58cm-1, 48.30%T
3602.23cm-1, 43.43%T
3910.89cm-1, 49.24%T
3300.49cm-1, 35.52%T
2914.50cm-1, 0.01%T
2853.25cm-1, 0.03%T
2661.93cm-1, 31.99%T
2841.58cm-1, 45.29%T
2020.15cm-1, 47.46%T
1898.32cm-1, 48.44%T
1637.10cm-1, 37.45%T
1564.96cm-1, 37.52%T
1466.99cm-1, 1.48%T
1407.21cm-1, 24.06%T
1374.94cm-1, 17.94%T
1248.32cm-1, 22.03%T
1065.96cm-1, 22.61%T
971.09cm-1, 28.75%T
807.07cm-1, 39.40%T
841.17cm-1, 35.75%T
725.53cm-1, 10.53%T
1115.14cm-1, 23.41%T
462.34cm-1, 43.97
Name
Description
isj128_1_1
Sample 266 By IIT Date Thursday, Septemb...


(C)
Control
ISJ128
Absorbance
0.582
0.3
0.378
0.14
0.237
0.06
0.237
0.09
KCBI
ECBI
VCBI
IDBI

◂**Fig. 8** FTIR spectra of polyethylene film used as control sample (**A**). Polyethylene film treated with *A. Faecalis* strain ISJ128 (**B**). KCBI, ECBI, VBI, and IDBI control sample and polyethylene film treated with ISJ128 (**C**)

of control (without bacteria) PE film exhibited a variety of peaks, indicating the complexity of LDPE film (Fig. 8A). In contrast, the polyethylene film treated with *A. faecalis* ISJ128 displayed alternation in the intensity of spectra in various regions (Fig. 8B). For control spectrum, the characteristic band was delegated at 729.01 cm^{-1} (C–H bending), 1,465.69 cm^{-1} (C–H bending), 1713.55 cm^{-1} (C=O stretching), 2,855 cm^{-1} (C–H stretching), and 2,916 cm^{-1} (C–H stretching). The shifting of existing peaks as well as the appearance of some new band at 1200–1400 cm^{-1} region of FTIR spectra, indicates the formation of new intermediates products (Fig. 8B). Bands at 2916 cm^{-1}, 1713.55 cm^{-1},1465.69 cm^{-1}, and 729.01 cm^{-1} in the control spectrum shifted to 2914.50 cm^{-1}, 1715.81 cm^{-1}, 1466.99 cm^{-1}, and 725.19 cm^{-1} in test spectrum, respectively, indicating formation of new peaks at various region during biodegradation. Our findings were corroborated by several previous investigations, for instance Das and Kumar, (2015) recorded chemical groups transformation in their polyethylene degradation conducted with *Bacillus amyloliquefaciens*. Da Luz et al. (2014) identified similar tendency of chemical groups transformation in oxo-biodegradable polymer with *Pleurotus ostreatus*. The variation in peak intensities of substantially all functional groups openly promotes the conformational shift in treated polyethylene film. Albertsson et al. (1987) earlier reported that the biological activity promotes the development of terminal double bonds (IR 905–915 cm^{-1}). An increase in ester carbonyl bond index (ECBI), keto carbonyl bond index (KCBI), vinyl carbonyl bond index (VBI), and internal double bond carbonyl index (IDBI) was clearly observed in the graph, calculated from FTIR spectra (Fig. 8C). Initially, carbonyl index was increased due to the oxidation of dissolved oxygen. Previous reports also confirm that besides the formation of carbonyl groups, the reduction of native bonds observed in the FTIR spectrum of polyethylene also serves as an indication that the polymer has been broken down to shorter chains (Rajandas et al., 2012). In biodegradation, enzymes catalyze a particular or series of responses that lead to different kinds of chemical transformations, like oxidation reduction, hydrolysis, esterification, and molecular inner conversion. Keto and ester carbonyls have been accounted for as significant groups within the sight of oxidoreductase. Our findings are consistent with those of Albertsson et al. (1987), who reported a rise in carbonyl index of low- and high-density polyethylene due to microbial activity.

Conclusion

Environmental pollution resulting from PE waste is a matter of global concern in the present time, which is specifically because of its inert properties that resist deterioration and degradation. Here, we have applied a multidisciplinary approach to assess efficient biodegradation of PE using indigenous microorganisms associated with partially degraded polyethylene collected from waste dump sites. It is concluded that *A. faecalis* ISJ128 develops substantial microbial biofilm over the LDPE surface causing its efficient biodegradation within a short span of time. As un-pretreated polyethylene was used for conducting biodegradation experiments, therefore, higher biodegradation might be possible to achieve by our bacterial strain for pretreated PE. Our study indicates that indigenous microbes from diverse environments such as waste disposal sites are promising LDPE degraders, and thus, may act as a suitable candidate to develop environment-friendly approach for the management of plastic waste. *Alcaligenes* sp. can be exploited as a novel strain to devise an eco-friendly tool toward plastic degradation. However, there is very limited information available on degradation of LDPE using large-scale studies. Further studies on isolation of enzymes responsible for biodegradation of plastic on molecular level will pave the way for large scale or industrial scale-up.

Author's contribution All authors contributed equally to perform the analysis and to collect experimental data under the supervision of KKG. The idea of performing this research and designing of methodology came from KKG and DD. Data analysis was conducted by KKG and DD. The draft manuscript was prepared by DD with the assistance of KKG, HC, KS, HDMC, and KKS. Manuscript was critically reviewed by KKG, APM, HDMC, and HC. All authors read and approved the final manuscript.

Funding This study did not received specific funding from public, private, or non-profit funding agencies.

Availability of data and materials Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

References

Albertsson, A. C., Andersson, S. O., & Karlsson, S. (1987). The mechanism of biodegradation of polyethylene. *Polymer Degradation and Stability, 18*(1), 73–87. https://doi.org/10.1016/0141-3910(87)90084-X

Ali, S. S., Elsamahy, T., Koutra, E., Kornaros, M., El-Sheekh, M., Abdelkarim, E. A., Zhu, D., & Sun, J. (2021). Degradation of conventional plastic wastes in the environment: A review on current status of knowledge and future perspectives of disposal. *Science of the Total Environment, 771*, 144719. https://doi.org/10.1016/j.scitotenv.2020.144719

Aravinthan, A., Arkatkar, A., Juwarkar, A. A., & Doble, M. (2016). Synergistic growth of Bacillus and Pseudomonas and its degradation potential on pretreated polypropylene. *Preparative Biochemistry and Biotechnology, 46*(2), 109–115. https://doi.org/10.1080/10826068.2014.985836

Arkatkar, A., Juwarkar, A. A., Bhaduri, S., Uppara, P. V., & Doble, M. (2010). Growth of Pseudomonas and Bacillus biofilms on pretreated polypropylene surface. *International Biodeterioration and Biodegradation, 64*(6), 530–536. https://doi.org/10.1016/j.ibiod.2010.06.002

Augusta, J., Müller, R. J., & Widdecke, H. (1993). A rapid evaluation plate-test for the biodegradability of plastics. *Applied microbiology and biotechnology*, *39*, 673–678.

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Screening of Bacillus strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. *Environmental Pollution, 231*, 1552–1559. https://doi.org/10.1016/j.envpol.2017.09.043

Awasthi, S., Srivastava, P., Singh, P., Tiwary, D., & Mishra, P. K. (2017). Biodegradation of thermally treated high-density polyethylene (HDPE) by *Klebsiella pneumoniae* CH001. *3 Biotech, 7*(5), 1–10. https://doi.org/10.1007/s13205-017-0959-3

Azeko, S. T., Etuk-Udo, G. A., Odusanya, O. S., Malatesta, K., Anuku, N., & Soboyejo, W. O. (2015). Biodegradation of linear low density polyethylene by *Serratia marcescens* subsp. marcescens and its cell free extracts. *Waste and Biomass Valorization, 6*(6), 1047–1057. https://doi.org/10.1007/s12649-015-9421-0

Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society b: Biological Sciences, 364*(1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Barra, R., Leonard, S.A. (2018). Plastics and the circular economy. In 54th Global Environment Facility Council Meeting, Da Nang, Viet Nam. June 24–26.

Beveridge, T. J. (2001). Use of the Gram stain in microbiology. *Biotechnic and Histochemistry, 76*(3), 111–118. https://doi.org/10.1080/bih.76.3.111.118

Bhatia, M., Girdhar, A., Tiwari, A., & Nayarisseri, A. (2014). Implications of a novel Pseudomonas species on low density polyethylene biodegradation: An in vitro to in silico approach. *Springerplus, 3*(1), 1–10. https://doi.org/10.1186/2193-1801-3-497

Briassoulis, D. (2004). An overview on the mechanical behaviour of biodegradable agricultural films. *Journal of Polymers and the Environment, 12*(2), 65–81. https://doi.org/10.1023/B:JOOE.0000010052.86786.ef

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environmental Science and Technology, 45*(21), 9175–9179. https://doi.org/10.1021/es201811s

Carmen, S. (2021). Microbial capability for the degradation of chemical additives present in petroleum-based plastic products: A review on current status and perspectives. *Journal of Hazardous Materials, 402*, 123534. https://doi.org/10.1016/j.jhazmat.2020.123534

Chandra, H., Srivastava, J., & Agarwal, R. K. (2016). *Fundamental techniques in microbiology*. John Publisher Pvt.

Cózar Cabañas, A., Echevarría Navas, F. M., González Gordillo, J. I., Irigoien, X., Úbeda, B., Hernández León, S., Palma, Á. T., Navarro, S., de García Lomas, J., Ruiz, A., de Fernández Puelles, M. L., & Duarte, C. M. (2014). Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences, 111*(28), 10239–10244. https://doi.org/10.1073/pnas.1314705111

da Luz, J. M. R., Paes, S. A., Bazzolli, D. M. S., Totola, M. R., Demuner, A. J., & Kasuya, M. C. M. (2014). Abiotic and biotic degradation of oxo-biodegradable plastic bags by *Pleurotus ostreatus*. *PLoS ONE, 9*(11), e107438. https://doi.org/10.1371/journal.pone.0107438

Das, M. P., & Kumar, S. (2015). An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech, 5*(1), 81–86. https://doi.org/10.1007/s13205-014-0205-1

de Sousa, F. D. B. (2021). The role of plastic concerning the sustainable development goals: The literature point of view. *Cleaner and Responsible Consumption, 3*, 100020. https://doi.org/10.1016/j.clrc.2021.100020

Duddu, M. K., Tripura, K. L., Guntuku, G., & Divya, D. S. (2015). Biodegradation of low density polyethylene (LDPE) by a new biosurfactant-producing thermophilic Streptomyces coelicoflavus NBRC 15399T. *African Journal of Biotechnology*, *14*(4), 327–340. https://doi.org/10.5897/AJB2014.14224

Farzi, A., Dehnad, A., & Fotouhi, A. F. (2019). Biodegradation of polyethylene terephthalate waste using Streptomyces species and kinetic modeling of the process. *Biocatalysis and Agricultural Biotechnology, 17*, 25–31. https://doi.org/10.1016/j.bcab.2018.11.002

Galgani, L., Beiras, R., Galgani, F., Panti, C., & Borja, A. (2019). Editorial: Impacts of marine litter. *Frontiers in Marine Science, 6*, 208. https://doi.org/10.3389/fmars.2019.00208

Gallo, F., Fossi, C., Weber, R., Santillo, D., Sousa, J., Ingram, I., Nadal, A., & Romano, D. (2018). Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. *Environmental Sciences Europe, 30*(1), 1–14. https://doi.org/10.1186/s12302-018-0139-z

Ganesan, P., Reegan, A. D., David, R. H. A., Gandhi, M. R., Paulraj, M. G., Al-Dhabi, N. A., & Ignacimuthu, S. (2017). Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India. *Alexandria Journal of Medicine, 53*(2), 101–110. https://doi.org/10.1016/j.ajme.2016.03.004

Garcia, J. M., & Robertson, M. L. (2017). The future of plastics recycling. *Science*, *358*(6365), 870–872.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3*(7), e1700782. https://doi.org/10.1126/sciadv.1700782

Gregory, M. R. (2009). Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1526), 2013–2025.

Gupta, K. K., & Devi, D. (2020). Characteristics investigation on biofilm formation and biodegradation activities of *Pseudomonas aeruginosa* strain ISJ14 colonizing low density polyethylene (LDPE) surface. *Heliyon, 6*, e04398. https://doi.org/10.1016/j.heliyon.2020.e04398

Gupta, K. K., Devi, D., & Rana, D. (2016). Isolation and screening of low density polyethylene (Ldpe) degrading bacterial strains from waste disposal sites. *World Journal of Pharmaceutical Research, 5*(11), 1633–1643.

Gupta, K. K., & Rana, D. (2021). Spectroscopic and chromatographic identification of bioprospecting bioactive compounds from cow feces: Antimicrobial and antioxidant activities evaluation of gut bacterium *Pseudomonas aeruginosa* KD155. *Biotechnology Reports, 29*, e00577. https://doi.org/10.1016/j.btre.2020.e00577

Gupta, K. K., Sharma, K. K., & Chandra, H. (2022). *Micrococcus luteus* strain CGK112 isolated from cow dung demonstrated efficient biofilm-forming ability and degradation potential toward high-density polyethylene (HDPE). *Archives of Microbiology, 204*(7), 1–13.

Hadad, D., Geresh, S., & Sivan, A. (2005). Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *Journal of Applied Microbiology, 98*(5), 1093–1100. https://doi.org/10.1111/j.1365-2672.2005.02553.x

Hamid, S. H. (2000). Ultraviolet-induced degradation of Ziegler–Natta and metallocene catalyzed polyethylenes. *Journal of Applied Polymer Science, 78*(9), 1591–1596. https://doi.org/10.1002/1097-4628(20001128)78:9%3c1591::AID-APP30%3e3.0.CO;2-A

Harshvardhan, K., & Jha, B. (2013). Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. *Marine Pollution Bulletin, 77*(1–2), 100–106. https://doi.org/10.1016/j.marpolbul.2013.10.025

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology*. Williams & Wilkins press.

Howard, G. T., & Hilliard, N. P. (1999). Use of Coomassie blue-polyurethane interaction inscreening of polyurethanase proteins andpolyurethanolytic bacteria. *International Biodeterioration and Biodegradation, 43*(1–2), 23–30. https://doi.org/10.1016/S0964-8305(98)00062-6

Igwo-Ezikpe, M. N., Gbenle, O. G., Ilori, M. O., Okpuzor, J., & Osuntoki, A. A. (2009). Evaluation of *Alcaligenes faecalis* degradation of chrysene and diesel oil with concomitant production of biosurfactant. *Research Journal of Environmental Toxicology, 3*(4), 159–169. https://doi.org/10.3923/rjet.2009.159.169

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Marine pollution. Plastic waste inputs from land into the ocean. *Science (new York N.y.), 347*(6223), 768–771. https://doi.org/10.1126/science.1260352

Karlsson, S., Ljungquist, O., & Albertsson, A. C. (1988). Biodegradation of polyethylene and the influence of surfactants. *Polymer Degradation and Stability, 21*(3), 237–250. https://doi.org/10.1016/0141-3910(88)90030-4

Kavitha, R., & Bhuvaneswari, V. (2021). Assessment of polyethylene degradation by biosurfactant producing ligninolytic bacterium. *Biodegradation*, *32*(5), 531–549. https://doi.org/10.1007/s10532-021-09949-8

Kong, L., Zhu, S., Zhu, L., Xie, H., Su, K., Yan, T., Wang, J., Wang, J., Wang, F., & Sun, F. (2013). Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. *Journal of Environmental Sciences, 25*(11), 2257–2264. https://doi.org/10.1016/S1001-0742(12)60288-5

Kumar, R., Verma, A., Shome, A., Sinha, R., Sinha, S., Jha, P. K., Kumar, R., Kumar, P., Das, S., Sharma, P. V., & Prasad, P. V. (2021). Impacts of plastic pollution on ecosystem services, sustainable development goals, and need to focus on circular economy and policy interventions. *Sustainability, 13*(17), 9963. https://doi.org/10.3390/su13179963

Kumar, S., Hatha, A. A. M., & Christi, K. S. (2007). Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *Revista De Biología Tropical, 55*(3–4), 777–786.

Kyaw, B. M., Champakalakshmi, R., Sakharkar, M. K., Lim, C. S., & Sakharkar, K. R. (2012). Biodegradation of low density polythene (LDPE) by Pseudomonas species. *Indian Journal of Microbiology, 52*(3), 411–419. https://doi.org/10.1007/s12088-012-0250-6

Lee, B., Pometto, A. L., III., Fratzke, A., & Bailey, T. B., Jr. (1991). Biodegradation of degradable plastic polyethylene by Phanerochaete and Streptomyces species. *Applied and Environmental Microbiology, 57*(3), 678–685. https://doi.org/10.1128/aem.57.3.678-685.1991

Mata-Lima, H., Alvino-Borba, A., Akamatsu, K., Incau, B., Jard, J., da Silva, A. B., & Morgado-Dias, F. (2016). Measuring an organization's performance: the road to defining sustainability indicators. *Environmental Quality Management*, *26*(2), 89–104.

Morét-Ferguson, S., Law, K. L., Proskurowski, G., Murphy, E. K., Peacock, E. E., & Reddy, C. M. (2010). The size, mass, and composition of plastic debris in the western North Atlantic Ocean. *Marine Pollution Bulletin, 60*(10), 1873–1878. https://doi.org/10.1016/j.marpolbul.2010.07.020

Nag, M., Lahiri, D., Dutta, B., Jadav, G., & Ray, R. R. (2021). Biodegradation of used polyethylene bags by a new marine strain of *Alcaligenes faecalis* LNDR-1. *Environmental Science and Pollution Research, 28*(30), 41365–41379. https://doi.org/10.1007/s11356-021-13704-0

Nanda, S., & Sahu, S. S. (2010). Biodegradability of polyethylene by Brevibacillus, Pseudomonas, and Rhodococcus spp. *New York Science Journal, 3*(7), 95–98.

Novotný, Č, Malachová, K., Adamus, G., Kwiecień, M., Lotti, N., Soccio, M., Verney, V., & Fava, F. (2018). Deterioration of irradiation/high-temperature pretreated, linear low-density polyethylene (LLDPE) by *Bacillus amyloliquefaciens*. *International Biodeterioration and Biodegradation, 132*, 259–267. https://doi.org/10.1016/j.ibiod.2018.04.014

Orr, I. G., Hadar, Y., & Sivan, A. (2004). Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *Applied Microbiology and Biotechnology, 65*(1), 97–104. https://doi.org/10.1007/s00253-004-1584-8

Patel, R., Nanda, S., & Dalai, A. K. (2021). Conversion of municipal solid waste to biofuels. In S. Nanda & N. V. Dai-Viet (Eds.), *Progressive thermochemical biorefining technologies* (pp. 43–61). CRC Press.

Pepper, I. L., Gerba, C. P., & Brendecke, J. W. (1995). *Environmental microbiology: A laboratory manual*. Academic Press.

Pramila, R., & Ramesh, K. V. (2015). Potential biodegradation of low density polyethylene (LDPE) by *Acinetobacter baumannii*. *African Journal of Bacteriology Research, 7*(3), 24–28. https://doi.org/10.5897/JBR2015.0152

Rajandas, H., Parimannan, S., Sathasivam, K., Ravichandran, M., & Yin, L. S. (2012). A novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation. *Polymer Testing*, *31*(8), 1094–1099. https://doi.org/10.1016/j.polymertesting.2012.07.015

Reddy, R. M. (2008). Impact of soil composting using municipal solid waste on biodegradation of plastics. *Indian Journal of Biotechnology, 7*, 235–239.

Restrepo-Flórez, J. M., Bassi, A., & Thompson, M. R. (2014). Microbial degradation and deterioration of polyethylene: A review. *International Biodeterioration and Biodegradation, 88*, 83–90. https://doi.org/10.1016/j.ibiod.2013.12.014

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports, 3*(1), 1–7. https://doi.org/10.1038/srep03263

Rosenberg, M., Gutnick, D., & Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters, 9*(1), 29–33. https://doi.org/10.1111/j.1574-6968.1980.tb05599.x

Ru, J., Huo, Y., & Yang, Y. (2020). Microbial degradation and valorization of plastic wastes. *Frontiers in Microbiology, 11*, 442. https://doi.org/10.3389/fmicb.2020.00442

Russell, J. R., Huang, J., Anand, P., Kucera, K., Sandoval, A. G., Dantzler, K. W., Hickman, D., Jee, J., Kimovec, F. M., Koppstein, D., Marks, D. H., Mittermiller, P. A., Núñez, S. J., Santiago, M., Townes, M. A., Vishnevetsky, M., Williams, N. E., Vargas, M. P., Boulanger, L. A., … Strobel, S. A. (2011). Biodegradation of polyester polyurethane by endophytic fungi. *Applied and Environmental Microbiology, 77*(17), 6076–6084. https://doi.org/10.1128/AEM.00521-11

Sarkhel, R., Sengupta, S., Das, P., & Bhowal, A. (2020). Comparative biodegradation study of polymer from plastic bottle waste using novel isolated bacteria and fungi from marine source. *Journal of Polymer Research, 27*(1), 1–8. https://doi.org/10.1007/s10965-019-1973-4

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances, 26*(3), 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005

Shah, A. A., Kato, S., Shintani, N., Kamini, N. R., & Nakajima-Kambe, T. (2014). Microbial degradation of aliphatic and aliphatic-aromatic co-polyesters. *Applied Microbiology and Biotechnology, 98*(8), 3437–3447. https://doi.org/10.1007/s00253-014-5558-1

Sivan, A., Szanto, M., & Pavlov, V. (2006). Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. *Applied Microbiology and Biotechnology, 72*(2), 346–352. https://doi.org/10.1007/s00253-005-0259-4

Skariyachan, S., Mahajanakatti, A. B., Grandhi, N. J., Prasanna, A., Sen, B., Sharma, N., Vasist, K. S., & Narayanappa, R. (2015). Environmental monitoring of bacterial contamination and antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River, a major drinking water source in Karnataka. *India. Environmental Monitoring and Assessment, 187*(5), 279. https://doi.org/10.1007/s10661-015-4488-4

Sudhakar, M., Doble, M., Murthy, P. S., & Venkatesan, R. (2008). Marine microbe-mediated biodegradation of low- and high-density polyethylenes. *International Biodeterioration and Biodegradation, 61*(3), 203–213. https://doi.org/10.1016/j.ibiod.2007.07.011

Tribedi, P., & Sil, A. K. (2013). Low-density polyethylene degradation by Pseudomonas sp. AKS2 biofilm. *Environmental Science and Pollution Research International, 20*(6), 4146–4153. https://doi.org/10.1007/s11356-012-1378-y

Velis, C. A. (2014). Plastic waste in marine litter: Action now and at the source. *Waste Management and Research: THe Journal of the International Solid Wastes and Public Cleansing Association, ISWA, 32*(4), 251–253. https://doi.org/10.1177/0734242X14528432

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A. D., Winther-Nielsen, M., & Reifferscheid, G. (2014). Microplastics in freshwater ecosystems: What we know and what we need to know. *Environmental Sciences Europe, 26*(1), 12. https://doi.org/10.1186/s12302-014-0012-7

Yang, C. Z., Yaniger, S. I., Jordan, V. C., Klein, D. J., & Bittner, G. D. (2011). Most plastic products release estrogenic chemicals: A potential health problem that can be

solved. *Environmental Health Perspectives, 119*(7), 989–996. https://doi.org/10.1289/ehp.1003220

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., & Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *The New England Journal of Medicine, 367*(19), 1814–1820. https://doi.org/10.1056/NEJMoa1211721

Zhang, H. H., Wang, Y., Zhao, C., Wang, J., & Zhang, X. L. (2017). Biodegradation of ochratoxin A by *Alcaligenes faecalis* isolated from soil. *Journal of Applied Microbiology, 123*(3), 661–668. https://doi.org/10.1111/jam.13537

Zhang, J., Gao, D., Li, Q., Zhao, Y., Li, L., Lin, H., Bi, Q., & Zhao, Y. (2020). Biodegradation of polyethylene microplastic particles by the fungus Aspergillus flavus from the guts of wax moth *Galleria mellonella*. *The Science of the Total Environment, 704*, 135931. https://doi.org/10.1016/j.scitotenv.2019.135931

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research, 31*(13), 3406–3415. https://doi.org/10.1093/nar/gkg595

Zylstra, E. R. (2013). Accumulation of wind-dispersed trash in desert environments. *Journal of Arid Environments, 89*, 13–15. https://doi.org/10.1016/j.jaridenv.2012.10.004