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GIS APPLIED TO SOIL-AGRICULTURAL HEALTH FOR ENVIRONMENTAL SUSTAINABILITY

Biological degradation of polyethylene terephthalate by rhizobacteria

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Abstract

In view of the growing demand for plastic products, an enormous proportion of plastic waste causing the biological issue is produced. Plants in collaboration with their rhizobacteria partners are also exposed to these contaminants. The study aims to determine the rhizobacterial ability to biodegrade PET plastic. We isolated the rhizobacteria capable of degrading the PET plastic in minimal salt media using it as a sole carbon source. The three rhizospheric isolates, namely *Priestia aryabhattai* VT 3.12 (GenBank accession No. OK135732.1), *Bacillus pseudomycoides* VT 3.15 (GenBank accession No. OK135733.1), and *Bacillus pumilus* VT 3.16 (GenBank accession No. OK1357324.1), showed the highest degradation percentage for PET sheet and powder. The biodegradation end products post 28 days for PET sheet and 18 days of PET powder were studied by Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), and scanning electron microscopy (SEM). Our results showed significant biodegradation of PET plastic, and the rate of degradation could account for over 65%. The present study proves soil rhizobacteria's potential and capabilities for efficient degradation of PET plastic occurring at the waste sites. It also implies that rhizobacteria could be beneficial in the remediation of PET waste in future applications.

Keywords PET plastic · Rhizobacteria · *Bacillus* · Biodegradation · HPLC · FTIR · SEM

Introduction

Microplastics (MPs) are the emerging potential contaminants having widespread occurrences in the ecosystem, including aquatic, atmospheric, and terrestrial (Singh et al., 2021a). They are usually plastic polymers with a particle

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size ranging from 1 µm to 5 mm. MPs are presumed toxic to humans and other living organisms due to their bioaccumulation and persistence properties. Pollution of microplastics is traditionally an irreversible process as they have low degradation rates and require sophisticated instrumentation to recover their particles. The demand for plastic is growing rapidly in different sectors of the industries. Polyethylene terephthalate (PET) is one of the widely applied plastic worldwide, with a consumption of 3 million tonnes in the European market in 2015.

PET pollution is likely an irreversible process with low degradation ability and is very hard to recover. However, the recovery of PET in the environment is highly challenging. The rates of reuse and recycling of PET are low, which leads to the mismanagement of plastic waste and causes accumulation in the environment. This disposed of plastic waste releases toxic compounds that cause soil and water pollution, and it also pollutes the air by releasing harmful emissions. The nanosized PET plastic causes several health diseases such as mental sickness, liver dysfunction, food poisoning, skin diseases, and respiratory diseases (Dhaka et al., 2022; Yaka et al., 2015). Hence, it menaces the health and lives of humans, animals, and plants.

Its increasing demand has brought a global issue regarding waste accumulation, which has remained in the environment for up to 100 years (Chen et al., 2020). For PET waste removal from the environment, several approaches have been proposed. It can be degraded by environmental erosion, biodegradation, thermal degradation, and photodegradation (Andrady, 2015). However, these methods are inclined more towards the demerits as compared to their merits except the biodegradation (Singh et al., 2022, 2021b). As recycling PET waste is of high cost, its practice is also limited (Awoyera and Adesina, 2020).

Biological degradation of PET is considered a green route as it has various merits such as minimizing PET waste, ecofriendly approach, and being easy to operate (Taniguchi et al., 2019; Zhang et al., 2017; Shoda et al., 2016). Biodegradation is preferred due to environmental and economic reasons (Farzi et al., 2017; Devi et al., 2015). To depolymerize PET, microbes release the extracellular enzymes and generate water-soluble intermediates. Microorganisms further utilize these intermediates for metabolism and degradation (Shah et al., 2008; Guebitz and Cavaco-Paulo, 2008). The presence of the ester group makes PET more resistant to biodegradation. So far, various PET degrading microorganisms have been reported which produces an extracellular enzyme, namely cutinase, lipase, PETase, protease, and esterase (Dąbrowska et al., 2021; Joo et al. 2018; Gong et al., 2018; Janczak et al. 2018a; Liu et al., 2018; Auta et al., 2017; Yoshida et al. 2016).

The use of plants for remediation purposes is a sustainable green technology that uses plants and their properties to assimilate, degrade, metabolize, transform, or remove harmful contaminants from various environments (Jha, 2020). In the rhizosphere, microorganisms externally and internally protect the plants and get benefit from root exudates and oxygen (Chamkhi et al., 2021). Rhizoremediation is among the most potent approach for remediation in polluted soil. Rhizoremediation is specific phytoremediation that involves plants and their associated rhizospheric microorganisms. It is the combination of phytoremediation and bioaugmentation with plant growth-promoting rhizobacteria. Although rhizoremediation can occur naturally, it can also be facilitated by inoculating soil with microorganisms capable of degrading environmental contaminants (Hussain et al., 2022). The rhizosphere microorganisms are present in polymer waste polluted and natural soils (Schwitzguebel 2017; Meena et al. 2017; Zhang et al., 2017). These microorganisms have a stimulating effect on plant development and growth (Janczak et al. 2014). These microbes can synthesize plant hormones as well as increase water and mineral substance uptake by plants (Janczak et al., 2018). Plastic degradation can be enhanced by the use of these microorganisms (Janczak et al. 2014). These rhizobacteria can survive in an anthropogenically degraded environment. Therefore, it is crucial to select the species of plant with rapid growth and biomass production with a large amount (Macci et al. 2013).

The purpose of this current research was to isolate the most effective bacterial strains for the degradation of PET. Three bacterial strains were isolated from the contaminated plastic sites with the ability to degrade the PET in natural conditions. This present study suggests the most cost-effective and green technique to degrade the PET under laboratory conditions.

Material and methods

Chemicals and media

Polyethylene terephthalate granules were obtained from Sigma-Aldrich. Nutrient agar and nutrient broth were of analytical grade used in this study. The mineral and minimal salt media both were of analytical grade. Methanol was an HPLC grade chemical.

Isolation of PET plastic degrading rhizobacteria

Soil samples were collected from the rhizospheric region of plants growing in the dumping area of Jalandhar, Punjab, in sterilized ziplock bags. Rhizospheric soil samples were collected at Focal point (coordinates 31° 21′ 36" N, 75° 34′ 26" E) near Lovely Professional University campus in district Jalandhar, Punjab, India. Rhizospheric soil samples were collected in sterile zip lock bags and bought to the

laboratory in cooler boxes. The soil samples were kept in a refrigerator at 4 °C till bacterial isolations. Enrichment technique was carried out by introducing nutrients in the form of carbon from PET that only allows the growth of an organism of interest for the efficient PET degradation. Two grams of rhizosphere soil was incubated in 100 mL fresh mineral salt media [ammonium chloride (NH_4Cl), 0.3 g/L; sodium chloride (NaCl), 0.15 g/L; potassium dihydrogen phosphate (KH_2PO_4), 0.9 g/L; disodium phosphate (Na_2HPO_4), 1.8 g/L; calcium chloride dehydrate ($CaCl_2.2H_2O$), 0.045 g/L; and magnesium sulfate monohydrate ($MgSO_4.H_2O$),0.15 g/L; pH adjusted to 7.0] supplemented with PET sheets (2 × 2 cm) as a sole carbon source and incubated at 37 °C in a rotatory shaker incubator at 120 rpm for 7 days (Farzi et al., 2019). Treated PET sheets were transferred to a fresh mineral salt medium with 10 mL of the culture after 7 days of incubation and allowed to incubate in a rotatory shaker incubator for another week under the same conditions. After four cycles of enrichment, 10 µl of the sample was plated on the minimal media with 1.5% agar for screening and incubated at 7 °C for 24 h to isolate the different bacterial strains (Kumar et al., 2020). The colonies of different morphology were subcultured onto nutrient agar plates.

Identification of the rhizobacteria

16 s rRNA sequencing was conducted at Yaazh Genomics (Chennai, India) for the identification of isolated strains. A 1.5-kb 16S rRNA gene fragment was amplified from the total DNA of each sample and sequenced using the universal primers 1492R (ACCTTGTTACGACTT) and 27 F to validate the identity of three isolates (AGAGTTTGATCMTGG CTCAG). The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The DNA was re-suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The blast was carried out to find the similarity, followed by a multiple sequence alignment tool (MUSCLE 3.7 Alignment tool). The partial 16S rRNA sequences were aligned with sequences deposited in the NCBI database, and phylogenetic analysis was performed with the MEGA XI software. Extraction of DNA from bacterial isolates, PCR amplification, construction of 16S rDNA clone libraries, sequencing, and phylogenetic analysis were all carried out according to thorough protocols described by the procedure mentioned in the literature. (Singh et al., 2019). All the three sequences were submitted to NCBI under the following accession numbers OK135732.1, OK135733.1, and OK135734.1.

16S ribosomal RNA (rRNA) gene analyses of the individual bacterial isolates were conducted at Yaazh Genomics. (Ahmadabad, India).

Bacterial biomass measurement

PET degrading strains were cultured in PET enriched minimal media at 28 °C in a rotatory shaking incubator at 150 rpm for 24 h. Bacterial cultures were centrifuged for 10 min at 6000 rpm, and the pellet was mixed with fresh media. The cell count was adjusted using 0.5 McFarland standards (10^7 cells/mL) for degradation studies.

Biodegradation experiments

PET powder

The granules were ground through a high-speed grinder having a mesh size of 300 µm (Gong et al., 2018). To achieve the size, the milled powder was passed through the mesh number 50 corresponding to the particle size of 300 µm. The minimal media of 20 mL was poured into the 50 mL culture tubes and sterilized by autoclave at 121 °C and 15 psi for 15 min. The strains were incubated in a minimal medium which consist of disodium phosphate (Na_2HPO_4), 7.0 g/L; ammonium chloride (NH_4Cl), 1.0 g/L; magnesium sulfate ($MgSO_4$), 0.25 g/L; potassium dihydrogen phosphate (KH_2PO_4), 3.0 g/L; and PET powder, 1.0 g/L (Kumar et al., 2020). Three replicate culture tubes were prepared. The rhizobacteria culture was grown in the nutrient broth overnight and diluted to an OD_{600} of 1. The overnight culture of 1 mL was added to each tube and was incubated at 30 °C at 120 rpm for 18 days. The PET powders were the only sole carbon source for rhizobacteria.

Treatment of PET films

The PET films were prepared by cutting the transparent PET bottles. Drinking PET bottles were cut into 2 × 2 cm square films, sterilized with 70% ethanol, and dried in a biosafety cabinet (Farzi et al., 2019). The overnight grown cultures in nutrient broth were added to the minimal media. The PET films were added to the medium as the only carbon source and kept for incubation at 30 °C at 120 rpm for 28 days. A flask without inoculation served as a sterile control. At the same time, the initial weight of the PET films was also measured (Kumar et al., 2020).

Determination of biodegradation rate by weight loss

For the biodegradation study, PET film weight was recorded before adding to the minimal media in the degradation experiment. After 28 days of the incubation period, the cells were completely removed from PET film using a washing procedure with aqueous sodium dodecyl sulfate (2% v/v) solution (Sivan et al., 2006). Then, PET film weight was again recorded. The biodegradation (X) extent was

calculated using Eq. (1) (Sarkhel et al., 2020). For PET residual extraction, 5 mL of toluene was added into the degradation suspension and centrifuged at 4000 rpm for 15 min. The organic phase was filtered using Whatman filter paper. Using an electric scale, the weight of filter paper was noted before and after filtration of an organic phase. To obtain the PET residual weight, the toluene residue was dried in an incubator at 50 °C (Farzi et al., 2019). Then, the biodegradation extent was calculated using the same equation mentioned below.

$$\chi_{(t)} = \frac{m_0 - m_t}{m_0} \times 100 \quad (1)$$

where, X_t = extent of degradation; m_o = initial weight, m_t = final weight.

High-performance liquid chromatography

To determine the degree of PET degradation, HPLC analysis was applied. The filtered liquid extracted from PET residual was used for HPLC analysis. The equipment used was Shimadzu LC-2010CHT equipped with an RP-C18 column and a manual sample injector. Separation was achieved with 80% methanol and 20% water as eluent at the wavelength of 240 nm (Gong et al., 2018). The flow rate was set to 1 mL/min, and the injection volume of 1 µL was performed. The column was maintained at a temperature of 40 °C.

Scanning electron microscopy

Plastic samples were soaked in 2% phosphate-buffered glutaraldehyde for cell fixation. For post-fixation, samples were submerged in 2% osmium tetra-oxide in an ice bath for 3 h. The samples were then dehydrated in graded EtOH (50, 75, and 100%) baths for 15 min each before undergoing critical point drying with CO_2. Dried samples were sputter-coated with gold using a Leica ACE600 Coater before imaging with a Zeiss ULTRA 55 FESEM operating at an accelerating voltage of 2 kV with 1 µs dwell time. Treated PET powder was dried completely in the oven at 50 °C for 4 h. SEM was performed to check the absorption of the rhizobacteria on PET powder.

Fourier transform infrared spectroscopy–attenuated total reflectance

Plastic PET strips were submerged in 30 mL 2% SDS and placed on the rotary shaker for 2 h (225 rpm, 37 °C) to remove biofilms, immersed in fresh diH_2O water and air-dried. A ThermoScientific iS5 infrared spectrometer and id7 diamond-ATR attachment was used to acquire spectra from 4000 to 450 cm^{-1} (4 cm^{-1} resolution) with Omnic software. Data were transformed using an N-B strong apodization and Mertz phase correction. Three areas were analyzed for each sample to obtain spectra that represent the average condition of the plastic surface. All infrared spectra were normalized by peak intensity to common C-H bending modes used for spectral normalization of these polymers: 1409 cm^{-1} for PET (Vague et al., 2019). The filtrate liquid sample was used for the analysis of FTIR.

Results and discussion

Isolation and screening of rhizobacteria

The individual isolates were grown in minimal media depleted of carbon where polyethylene terephthalate acted as the primary carbon source. Molecular characterization (16 s rRNA) of isolated strains were submitted to the National Center for Biotechnology Information (NCBI). The three sequences unveil the highest similarity (> 99%) to that of *Priestia aryabhattai* VT 3.12 (GenBank accession No. OK135732.1), *Bacillus pseudomycoides* VT 3.15(GenBank accession No. OK135733.1), and *Bacillus pumilus* VT 3.16 (GenBank accession No. OK1357324.1) respectively. The phylogenetic tree of all the isolated strains was constructed using MEGA XI software with 1000 bootstraps (Suppl. Figure 1).

Determination of biodegradation rate

Biodegradation of PET sheet by VT3.12, VT3.15, and VT3.16isolates individually was performed for 28 days. After the incubation, weight loss was calculated using the same degradation formula mentioned above. It was found that the degradation rates were 40, 36, and 32% shown by VT3.12, VT3.15, and VT3.16 isolates (Table 1). Further biodegradation of PET powder of 300 µm particle sizes was carried out for 18 days with VT3.12, VT3.15, and VT3.16 isolates. At the last date of incubation, VT3.12, VT3.15, and VT3.16 showed the 69%, 66%, and 64% degradation rates of PET powder, respectively (Table. 2). Comparison of powdered samples biodegradation with sheet sample proves that powdering the PET is highly effective due to the availability of a larger surface area. Therefore, the high plastic degradation rates could be obtained by powdering the PET plastic.

Biodegradation analysis

SEM analysis

Biofilm formation is essential for the colonization of plastics by microorganisms, and without them, plastic cannot

be effectively degraded (Sivan et al., 2006). It allows for the visualization of the biofilm and bacterial colonization. The different samples were investigated by SEM analysis at the end of the experiment, and micrographs of the different PET samples are represented in Figs. 1 and 2. As can be seen, rhizobacteria were able to attach to the surface of the PET sheet and forms the biofilm, which is necessary for the biodegradation process (Fig. 2). These figures are in accordance with previous studies that demonstrate bacteria adhesion on PET surfaces (Demirkan et al., 2020). SEM was also done to check the rhizobacteria absorption on PET powder. The SEM images showed a significant difference between the native and degraded PET powder seen in Fig. 1. The virgin PET appears smooth, and there are no bacteria on the

Table 1 Observed weight loss in different polyethylene terephthalate during biodegradation processes

Polyethylene terephthalate	Symbol	Before treatment (g)	After treatment (g)	Standard deviation (SD)	Standard error mean (SEM)	Degradation %
Sheet	VT3.12	0.25	0.15	0.0010	0.0006	40.00
	VT3.15	0.25	0.16	0.0010	0.0006	36.00
	VT3.16	0.25	0.17	0.0010	0.0006	32.00
Powder	VT3.12	1.04	0.32	0.0021	0.0012	69.23
	VT3.15	1.05	0.35	0.0021	0.0012	66.66
	VT3.16	1.03	0.37	0.0012	0.0007	64.07

Table 2 HPLC data displaying degradation percentage of PET powder

S.No	Symbol	Retention time	Area at 0 day	Area after 18 days	Degradation efficiency (%)
1	Control	10.85	30,311,898 ± 21	30,311,898 ± 2458	00.00
2	VT3.12	10.84	30,311,898 ± 24	9,399,614 ± 2016	68.99
3	VT3.15	10.82	30,311,898 ± 20	10,005,852 ± 1728	66.99
4	VT3.16	10.83	30,311,898 ± 25	10,915,209 ± 2191	63.99

The symbol ± represent the standard deviation (SD) of the means ($n = 3$)

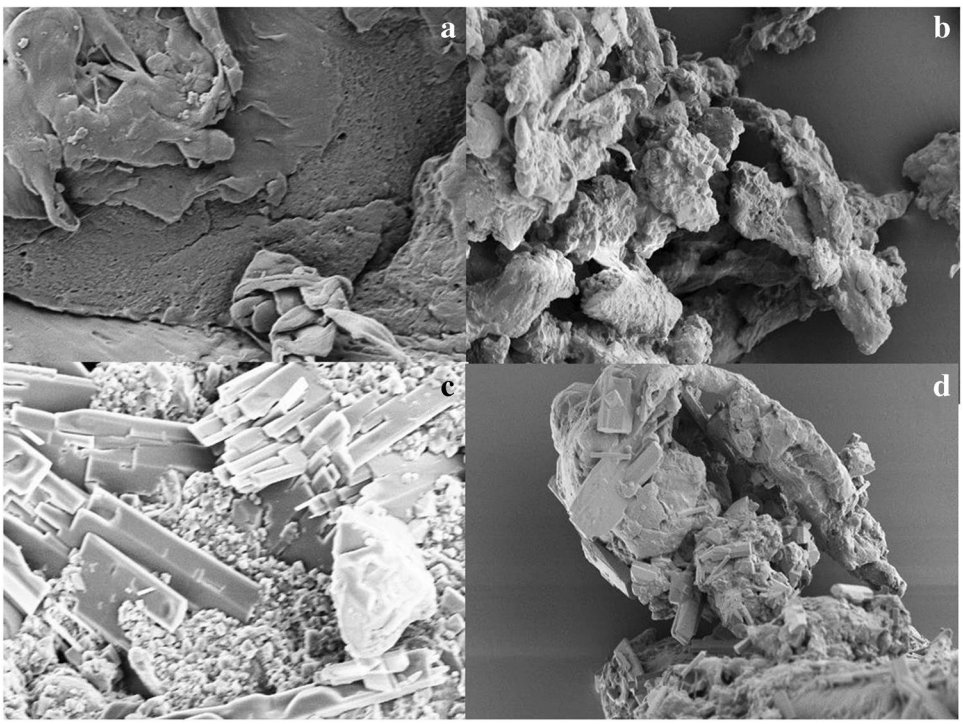

Fig. 1 SEM micrographs of PET powder after incubation in 18 days in carbon-free media inoculated with individual isolates. (**a**) Control-without treatment (original magnification 10.70 KX); (**b**) VT3.12 (original magnification 2.15 KX); (**c**) VT3.15 (original magnification 10.00 KX); (**d**) VT3.16 (original magnification 2.76 KX)

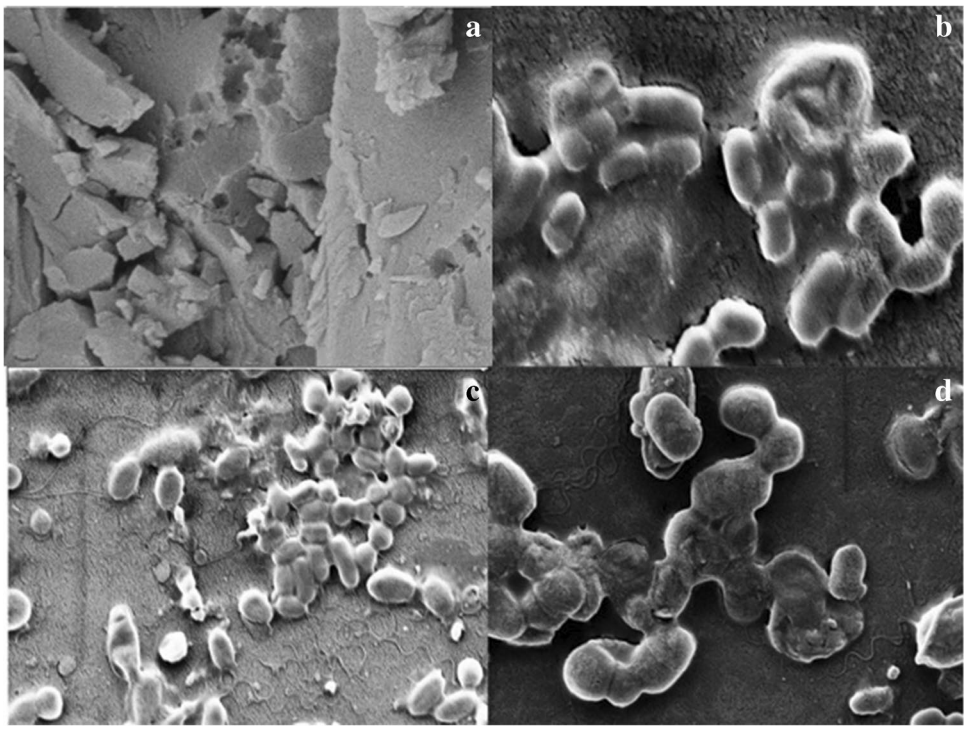

Fig. 2 SEM micrographs indicating biofilm formation on PET sheet after incubation of 28 days in carbon-free media inoculated with individual isolates. (**a**) Control- without treatment; (**b**) VT3.12; (**c**) VT3.15; (**d**) VT3.16

surface, whereas the incubated sheets appeared uneven on the surface, which was likely the result of microbes.

HPLC analysis

High-performance liquid chromatography (HPLC) detected the extent of PET powder biodegradation. In this study, PET powder was only the sole carbon source and energy of the culture medium of PET incubated with VT3.12, VT3.15, and VT3.16. The shift in the peak area indicates the degradation of PET powder (Table 2). The degradation percentage was calculated using the degradation percentage formula. The peak area decreases of treated PET powder samples compared to the control, whereas the peak retention time remains similar. Therefore, this change in the area confirms the breakdown of PET polymer into its monomers. Out of three isolates, VT3.12 showed the high degradation activity for PET powder, and the degradation rate was all above 69%, indicating that rhizobacteria had the capability of degrading the PET plastic that existed in the environment.

FTIR-ATR spectroscopic analysis

For both samples (powdered and sheet PET), the FTIR measurements were taken at 18- and 28-day intervals during degradation. The peaks or bands of absorption present in pristine PET, which later disappeared with the degree of degradation, proved the efficacy of the degradation process (Umamaheswari and Murali, 2013). The FTIR spectroscopic studies are conducted in the 400 to 4000 cm^{-1} range. The peak at 866 cm^{-1} refers to the C = C bond stretching (aromatic), which in the case of the powder PET samples is seen to become less intense with time slowly. This might be indicative of oxidation and reduction of the unsaturation present in the PET sample. The peaks around 1412 cm^{-1} and 1341 cm^{-1} refer to C-H bond stretching (methylene group), which becomes less intense for both samples of PET with time indicating successful degradation of the aliphatic carbon backbone (Gu and Gu, 2005). The bands at 1245 cm^{-1} and 1065 cm^{-1} correspond to C-O bond stretching (ether group formation). These bands are present in the pristine PET samples, and the samples are degraded for 12 h. They, however, disappear in the samples exposed for longer durations.

The absorption bands at 3310 cm^{-1} have been attributed to O–H bond stretching, which appears in the case of the powder sample degraded at 12 h. This absorption band is seen to almost slowly disappear with the increase in exposure time. This introduction of the O–H bonds can be indicative of efficient intermolecular hydrogen bonding, indicating successful degradation of PET (Alzuhairi et al., 2016). Interestingly, this band does not appear for the sheet samples, which could also indicate a higher degree of hydroxylation in the powder samples because of more exposure due to higher surface area (Fig. 3). The peak around 1715 cm^{-1} can be attributed to C = O bond stretching, which is seen to become less intense with the increase in time and almost disappears in the sample exposed for 16 h. This pattern is

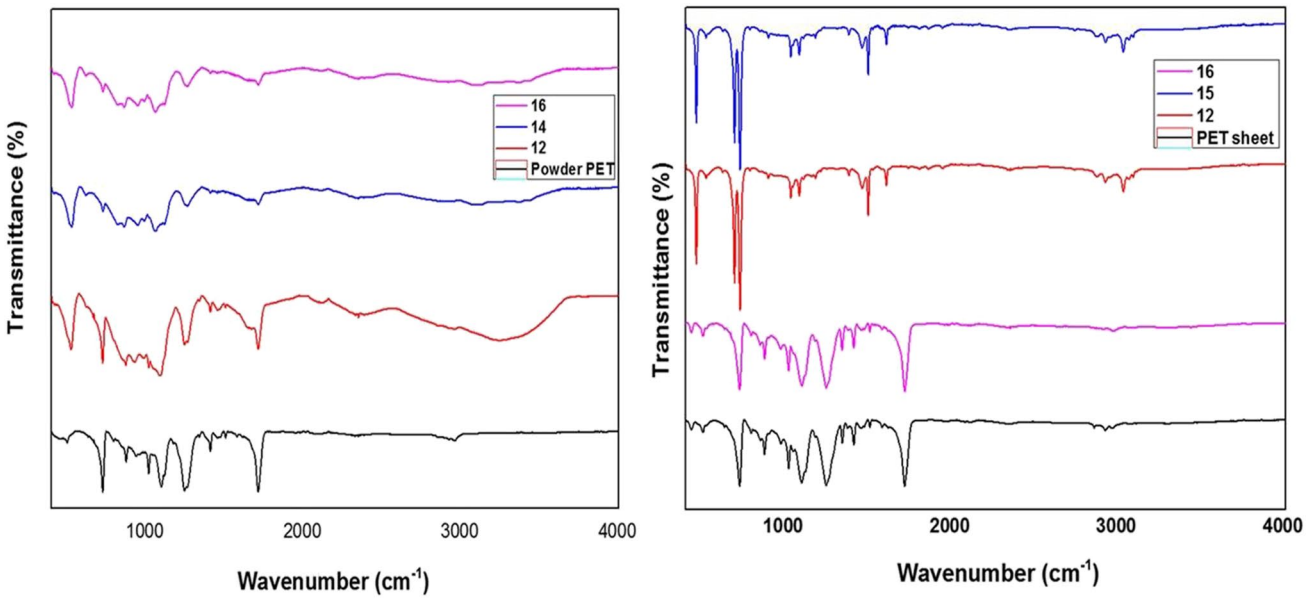

Fig. 3 FTIR spectra of 18 days PET powder inoculated with individual isolates VT3.12, VT3.15, VT3.16 in minimal salt media (left) and PET sheet (right)

noticed in both cases. This indicates the degradation of carbonyl groups present in the system. Our FTIR results are consistent with the finding of Markandan et al. (2020), in which *Alcaligenes faecalis* was used for the degradation of PET. Their results revealed that FTIR peaks become broader in the region 4000 to 3000 cm^{-1} of UV-treated PET flakes. The adherence and colonization of *A. faecalis* on the surface of Pet flakes were also observed.

Our results showed significant biodegradation of PET plastic, and the rate of degradation could amount to over 65%. Therefore, they could help in the remediation of environmental pollution caused by PET plastic. The cleavage of bonds like C-O, C-H, C = O, C = C, and the introduction of hydroxyl groups in the case of powdered samples can be noticed. This provides evidence of considerable degradation of the PET samples in both cases. The powdered sample is seen to have a greater degree of cleavage of the chemical bonds mentioned. Similar studies were reported by Gong et al. (2018), in which they used *Aeromonas* strain for the depolymerization and assimilation of PET. They have engineered the strain *Comamonas testosterone* F4 and found that the granular crystallinity of PET was increased by 1.81%, and still sufficient amorphous region is available for the degradation. Terephthalic acid (TA), mono (2-hydroxyethyl), terephthalate (MHET), bis (2-hydroxyethylterephthalate) (BHET), muconic acid (MA), and benzoic acid (BA) were the main products formed after the depolymerization of PET. Another study conducted by Yoshida et al (2016) reported the isolation of *Ideonella sakaiensis* 201-F6 bacteria for the biodegradation of PET from the recycling site of the PET bottle. They incubated the PET film at 30 °C for 6 weeks. They have used the PET film of low crystallinity of 1.9% as the sole carbon source. Their results revealed that this bacterium degrades the PET by 75% by secreting the enzyme PETase and MHETase. This bacterium adheres to the surface of PET and releases the PETase enzyme to target the polymer. Further, it produces MHETase enzyme which converts the intermediate product MHET into TPA and ethylene glycol formed after the breakdown of PET. Similar studies were reported by different investigators for the biodegradation of PET using different bacterial species (Liu et al., 2018; Taniguchi et al., 2019; Gao and Sun, 2021; Janczak et al., 2018b; Farzi et al., 2019) and the comparison of the biodegradation studies with present study is presented in Table 3. As the all three selected bacterial strains exhibited the ability to grow and survive on PET powder and sheets alone without any carbon or other energy source. This quality makes them ideally suitable for biodegradation of PET sheets and powder under natural conditions. Thus, the selected strain plays a possible role in bioremediation the areas where plastic pollution is abundant. Our study confirms that the strains were capable to grow and degrade PET residues under various conditions by a high percentage. For the control experiment, *E. coli* was used. From the first day, no change in the growth of the *E. coli* was observed. This is due to the deficiency of nutrients in the minimal media which does not favor the growth of *E. coli*.

Table 3 Comparison of different bacterial performance with previously reported bio-degradation of powder and sheets

S.No	Bacteria	Biodegradation extent (%)		Time period	References
		PET sheet	**PET powder**		
1	*Acinetobacter baumannii*	27.363	–	28 days	Hussein et al., 2018
2	*Ideonella sakaiensis* 201-f6	58	–	42 days	Yoshida et al., 2016
3	*Thermobifida fusca* KW3 (LC-cutinase and TfCut2)	20.4	–	24 h	Barth et al., 2016
4	*Saccharomonos poraviridis*Cut190	27	–	3 days	Kawai et al., 2014
5	*Streptomyces* species	–	49.2 (500 µm) 57.4 (420 µm) 62.4 (300 µm) 68.8 (212 µm)	18 days	Farziet al., 2019
6	*Bacillus subtilis* (B05) *Bacillus halodurans* (B03) *Bacillus okuhidensis* (B08) *Bacillus pumilus* (B10)	–	0.3 0.2 0.1 0.4	15 days	Chaveset al., 2018
7	Consortium No. 46	75	–	–	Taniguchi et al., 2019
8	*Rhococcus* sp. SSM1	30.52	–	132 h	Kumar et al., 2020
9	*Vibrio* sp. PD6	35%	–	6 weeks	Sarkhel et al., 2020
10	ITP3.4 (*Bacillus* sp.)	3.41	–	30 days	Chandra Kesi et al., 2020
11	*Bacillus cereus* *Bacillus gottheilii*	6.6 3	–	40	Roager and Sonnenschein, 2019
12	*Pseudomonas sp.*	0.6	–	100 days	Taghavi et al., 2021
13	Consortium (*Bacillus cereus*) SEHD031MH and *Agromyces mediolanus* PNP3	–	17	168 days	Torena et al., 2021

Conclusions

In this study, biodegradation of PET plastic waste with isolated strains from the rhizospheric soil was examined on a laboratory scale. Our findings represent that PET polymer can be degraded by these rhizobacteria with high efficiency. Three bacterial strains *Priestia aryabhattai* VT 3.12 (GenBank accession No. OK135732.1), *Bacillus pseudomycoides* VT 3.15 (GenBank accession No. OK135733.1), and *Bacillus pumilus* VT 3.16 (GenBank accession No. OK1357324.1) have shown the capability of producing biofilm on the surface of the polymer and high metabolic activity. The degraded PET films and powder have been determined by SEM, FTIR, HPLC, and weight loss methods. It was found that VT3.12 isolate degraded the PET sheet and powder more as compared to the other isolates. The degradation rate of PET sheet and powder was 40% and 69% respectively shown by VT3.12. It was also found that PET powder or small particles can be degraded more rapidly as compared to the biodegradation results of the PET sheet. Due to the availability of low surface area available for bacterial attachment, the PET sheets showed a lower biodegradation rate. Surface characterization also showed the presence of microbes on sheets which results in the damage of the surface. Therefore, all these strains have been used in future to process the plastic waste to minimize the damage to the environment by PET. Thus, microbial mineralization is a promising and cost-effective method for the degradation of the types of plastic polymers. Therefore, it is concluded that this technology is a promising solution for the degradation of composite and polymeric materials.

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Author contribution VD and SS have contributed in conception, exploring literature, and writing. JS and VD designed and acquired the entire concept and guided the authors. VD, SS, PCR, JS, TSSKN, SK, RP, and PCR have made the contributions including drafting and data verification under the leadership of Prof. JS (corresponding author). All authors read and approved the final manuscript.

Data availability The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Declarations

This article does not contain any studies with animals performed by any of the authors.

Consent for publication On behalf of the authors, Prof. Joginder Singh has been given authorization to submit and correspond.

Conflict of interest The authors declare no competing interests.

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