Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i2.3197

Research Article

Evaluation of *Bacillus*-Associated Polyethylene Terephthalate (PET) Surfaces For Biodegradation

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ABSTRACT

The escalating threat of polyethylene terephthalate (PET) microplastic pollution necessitates an urgent environmentally friendly approach. This study explores the potential of local bacterial isolates to biodegrade PET microplastics. Bacteria isolated from the surface of PET plastic bottles taken from Dengkil Inert Waste Landfill were screened for esterase activity and biofilm formation. PET degradation was assessed through mass weight reduction, scanning electron microscope (SEM) analysis for morphological changes, and Fourier-transform infrared spectroscopy (FT-IR) for chemical structure alterations. Two of the five selected isolates were positive for producing esterase and biofilm ability as well as biodegrade PET leading to a 2.04% - 2.08% degradation (20 days incubation). FT-IR indicated changes in PET chemical structure, particularly in C-H, C=O, and C-O bonds, while SEM revealed morphological changes such as cracks and holes on the PET surface. Identification through 16S rRNA sequencing indicated bacterial isolates are *Bacillus paramycoides* DIWL 1, *Bacillus cereus* DIWL 2, *Bacillus safensis* DIWL 3, *Bacillus luciferensis* DIWL 4, and *Bacillus cereus* DIWL 5. The results of this study can be used for further research on the potential of local isolates in the decomposition of PET microplastics which in turn can be used to develop a sustainable and cost-effective microplastic treatment (PET) technology.

Key words: *Bacillus*, biodegradation, microplastic, PET, synthetic polymers

INTRODUCTION

Plastic is one of the most well-known and widely used synthetic polymer materials worldwide. The nature of cheap, lightweight, durable, corrosion-resistant, and insulating, causes plastics to be in very high demand from consumers (Farzi *et al*., 2019; Chen *et al*., 2021). Plastics are categorized into thermosetting polymers and thermoplastics where the thermosetting plastic will not melt when heated and cannot be structurally modified, while thermoplastic is a plastic that can melt when heated and harden when cooled (Issac & Kandasubramanian 2021). Polyethylene terephthalate (PET) is one example of thermoplastics and is widely used to manufacture plastic water bottles, food wrappers, automotive tools, and electronic devices (Webb *et al*., 2013; Maity *et al*., 2021). PET is considered a major ecological pollution threat nowadays due to its increasing use.

The increase in plastic production has led to an abundance of plastic waste disposal. The degradation of accumulated plastic waste will form microplastics which are plastic particles with a diameter of less than 5 mm (Noik *et al*., 2015; Xuyang *et al*., 2025). The presence of microplastics in the environment is alarming because they can be ingested and introduced into the biological systems of a diverse range of organisms, ranging from herbivores and secondary consumers to predators at higher trophic levels, including microorganisms, planktons, benthic invertebrates, fish, deep ocean biota and larger mammals, causing neurotoxicity and genotoxicity, as well as reduced feeding, filtration, survival and reproductive abilities. These consequences reduce both the quantity and quality of food available for humans and other aquatic species (Amelia *et al*., 2021).

Since PET microplastics cause perilous pollution, it is necessary to find an efficient and environmentally friendly method to mitigate its pollution from the environment. PET being polyester in nature and having aromatic groups on its chemical structure makes PET more difficult to decompose than other polymers (Farzi *et al*., 2019). Apart from non-biological decomposition processes - such as photooxidation (uses light) and thermal decomposition (uses heat) - there is also biological decomposition carried out by microorganisms (Qi *et al*., 2022). In this case, biological decomposition is preferred due to economic factors, as it helps reduce pollution without harming the environment (Yuan *et al*., 2020).

Studies have shown that microorganisms can colonize microplastic surfaces (PET) and form biofilms producing enzymes to alter the chemical structure of polymers to be decomposed (Webb *et al*., 2013; Srivastava *et al*., 2024). Some of the most well-studied PET-degrading bacteria belong to the genera *Thermobifida*, *Ideonella*, *Pseudomonas,* and *Bacillus* which are isolated mainly from soil (Taniguchi *et al*., 2019; Roberts *et al*., 2020;). However, exploration of bacteria associated with PET surface is still scarce and can be traversed in facilitating biodegradation of PET without imposing adverse impacts. The core objective

Article History
Accepted: 28 May 2025
First version online: 30 June 2025
Cite This Article:
Khalil, K., Mohamad Desa, A.L., Ruslan, N.A., Mohd Yasin, N.H., Anuar, F.H., Aqma, W.S. 2025. Evaluation of Bacillus-associated polyethylene terephthalate (PET) surfaces for biodegradation. Malaysian Applied Biology, 54(2): 19-27. https://doi.org/10.55230/mabjournal.v54i2.3197

of this research is to evaluate the biodegradation potential of the local bacterial isolates associated with PET plastic surface sampled from inert waste landfills through Fourier Transform Infrared spectroscopic (FT-IR) and Scanning Electron Microscopic (FE-SEM) analyses.

MATERIALS AND METHODS

Sampling site

In this study, PET plastic bottles were sampled from the closed landfill (last operation in 2018) in Dengkil Inert Waste Landfill, Dengkil, Selangor, Malaysia (2.8697451, 101.6483268) (Figure 1). The condition of the PET bottles was still intact but apparent scratches and biofilm on the surface of the bottle. The PET bottles were collected and kept in a sterile sealer bag and stored at 4°C until further usage.

Fig. 1. Location of PET bottle waste sampling in Dengkil Inert Waste Landfill (red triangle).

Isolation of PET-degrading bacteria

The PET plastic bottles collected from the landfill were cut into small pieces (2 cm × 1.5 cm), washed with distilled water to remove the soil residue attached to the PET plastic, and transferred into 10 mL of normal saline (0.9% w/v). The sample was vortexed for 5 min and followed by a serial dilution from 10^{-1} to 10^{-3}, a total of 0.1 mL of samples from all dilutions was transferred on nutrient agar (Oxoid Ltd, United Kingdom) using spread plate technique and subsequently incubated at 30°C for 48 hr. Bacterial colonies with distinct morphology, pigmentation, and size were selected and subcultured on a fresh nutrient agar to obtain a single colony and incubated at 30°C for 24 hr. All experiments were conducted with an aseptic technique with three biological replications (Puglisi *et al*., 2019).

Screening of esterase activity

The screening process of esterase activity was performed using 1% and 2% (w/v) tributyrin agar. The bacterial colonies were streaked on the agar and incubated at 30°C for 2 to 5 days and the formation of a halo zone was observed as an indicator of esterase production and recorded as a positive result (Molitor *et al*., 2019).

Biofilms assay

Biofilm formation assays were performed according to Dąbrowska *et al*., (2021) with modifications and the experiment was performed in triplicate. The selected isolates were inoculated in 10 mL of nutrient broth and incubated at 30°C for 24 hr. After incubation, the concentration of the bacterial suspension was adjusted to a McFarland 0.5 (Abs 0.7 - 1.0_{600nm}). A total of 300 µL bacterial suspension was inoculated in a 1.5 mL tube with PET film (size: 6 mm × 6 mm, thickness 0.25 mm, Goodfellow, Sigma-Aldrich, USA; pre-sterilized with 70% alcohol & UV light for 15 min). While the negative control tubes contained only nutrient broth (Oxoid Ltd, United Kingdom) and PET film. All tubes were incubated at 30°C overnight. After incubation, the PET film was removed and placed in a 24-well plate and rinsed with 200 µL of PBS twice. Next, the film was stained with 100 µL of 1% crystal violet (w/v) and left for 15 min. Subsequently, the film was rinsed with 200 µL of PBS (Oxoid Ltd, United Kingdom) several times and allowed to dry at room temperature. Later, the PET film was transferred into a 96-well plate (flat bottom; SPL Life Science,

Korea) and a total of 200 µL of 99% ethanol was introduced into the well for measuring the absorption value at 595 nm using a microplate reader (Agilent BioTek Epoch). The biofilm index was calculated using the following formula and the biofilm-producing bacterial classification table was determined according to Kirmusaoğlu (2019).

OD isolates = Average OD of 0Dc	–	Equation 1
Cut Off OD (ODc) = Average OD negative control+ A	–	Equation 2
A = 3 × standard deviation (SD) of negative control	–	Equation 3

Biodegradation assay of PET granules

The screening process of PET biodegradation assay was conducted using the modified method of Auta *et al*. (2017). The initial weight of PET granules (Sigma-Aldrich; size: 2.5mm × 4mm; density: 1.68 g/mL; 30% glass particle as a reinforcer) was weighed and sterilized with 70% alcohol and followed by sterilization under UV light for 2 hr. Selected isolates that had been cultured in 10 mL of nutrient broth for 24 hr were centrifuged at 6,000 rpm for 15 min and pellets were taken up. Next, the pellets were resuspended into 4 mL of normal saline solution (0.9%, w/v) and the cell concentration was adjusted to McFarland 0.5 (Abs 0.07 - 0.1_{600nm}). Flasks containing liquid carbon-free basal medium (LCFBM) together with PET granules weighing 0.048 g to 0.049 g were prepared according to Yang *et al*. (2014) and divided into two sets. LCFBM prepared containing (per 1L) 0.7 g of KH_2PO_4, 0.7 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4SO_4, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$ and 0.001 g of $MnSO_4 \cdot H_2O$. The first set contained 10% (v/v) of each selected bacterial isolate and 18 mL of LCFBM and PET granules. While the second set acts as a control containing LCFBM and PET granules only. The experiments were done in duplicate. All conical flasks were incubated at 30°C and 150 rpm for 20 days. After 20 days, PET granules were taken and washed using 70% alcohol and subsequently dried in a hot air oven at 50°C overnight. Then the weight of the PET granules was weighed to obtain the final weight. Finally, the weight percentage of PET microplastic loss was calculated using the following calculations:

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

Analysis of PET surface

Changes in the carbonyl index of the PET granules were analyzed using FTIR spectroscopy in the frequency range of 4000–500 cm^{-1}. This analysis was performed on all PET granules treated with bacterial isolation and untreated PET (Auta *et al*., 2017). Structural changes in PET granules were analyzed using a scanning electron microscope (SEM) at 2000 – 5000× on all PET granules treated with bacterial isolates and untreated PET.

Identification of isolated bacteria

Identification of bacterial isolates was carried out based on 16S rRNA sequencing (Charnock 2021). The hot boiling method (Pui *et al*., 2011) was performed on the selected isolates to provide the DNA template. The reaction mixture for PCR contained 25 µL of GoTaq® Green Master Mix (Promega), 1 µL DNA template, 1 µL of each of primers 27F (5'AGA GTT TGA TCM TGG CTC AG 3'), 1492R (5' TAC GGY TAC CTT GTTACG ACTT 3') and PCR-grade water up to 50 µL. PCR conditions were: initial denaturation at 95°C (10 min) followed by 35 cycles of 95°C/30 s, 50°C/30 s and 72°C/min. This was followed by a final elongation of 72°C /5 min.

Statistical analysis

Analysis of data was performed using one-way ANOVA from Minitab 19 (Auta *et al*., 2017).

RESULTS AND DISCUSSION

Bacterial isolation and screening of esterase activity

A total of 16 bacterial isolates were successfully isolated from the surface of PET plastic bottles by the spread plate method. Screening for esterase activity resulted in 5/16 (designated as DIWL 1, DIWL 2, DIWL 3, DIWL 4 & DIWL 5) exhibiting clear zones showing the ability to hydrolyze tributyrin at 1% and 2% (v/v) after incubation for 2 days (Figure 2). Esterase is among the major class of enzymes involved in plastic degradation (Charnock 2021). The ability of bacteria to produce esterase indicates the potential to degrade PET (Sharon & Sharon 2012).

Tributyrin as a substrate, is a short-chain fatty acid triglyceride hydrolysed by lipolytic enzymes such as esterases, lipases, and polyesterases produced by bacteria. Triglycerides will be hydrolyzed to glycerol and fatty acids causing bacteria to use this end-product as a source of carbon and energy for bacterial growth by resulting in the formation of clear zones (Molitor *et al*., 2019). In this study, *Bacillus* species isolated from the surface of a PET bottle obtained from Dengkil Inert Waste Landfill has shown the ability to produce esterase when incubated for 2 days at 30°C. It is highly possible as *Bacillus* is abundant in soil and to reduce waste, the bacterium can utilize the components in municipal solid waste and break them down into inorganic salts, water, and gases (Wang *et al*., 2022). Roberts *et al*. (2020) reported that *B. thuringiensis* and *B. albus* produce esterase and exhibit the ability to degrade PET.

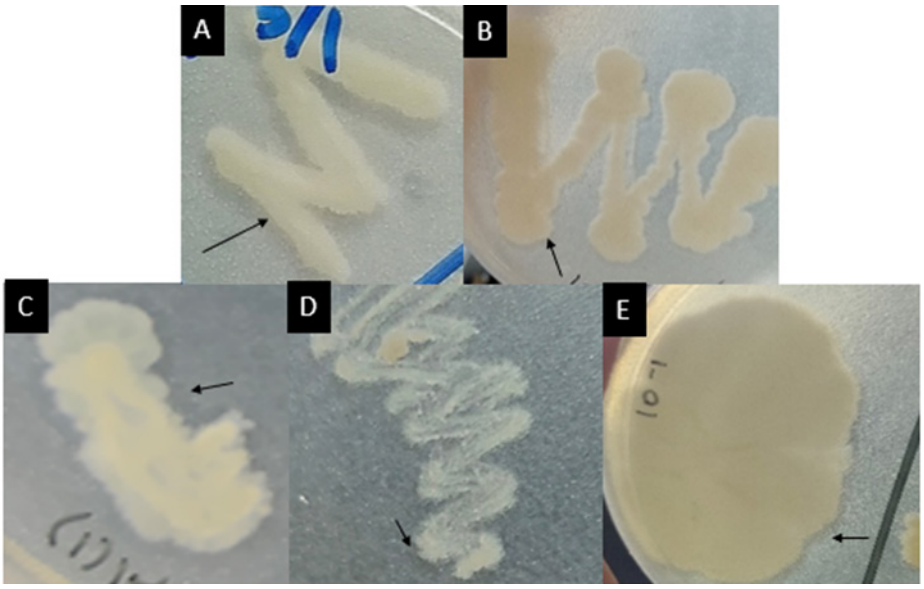

Fig. 2. Screening of esterase activity using tributyrin as substrate. Arrow showed the formation of a halo zone near the colonies indicating production of esterase by bacterial isolates. A) DIWL 1, B) DIWL 2, C) DIWL 3, D) DIWL 4, and E) DIWL 5 respectively on tributyrin agar plate incubated for 2 days (A-E).

Biofilm formation on PET surface

Biofilms play an important role in plastic decomposition occurs efficiently by bacteria (Dąbrowska *et al*., 2021). The current study recorded the presence of biofilm formation by bacteria isolates on the surface of PET after 24-hr incubation at 30°C. Isolates DIWL 1 and DIWL 2 showed the ability to form biofilm whereas, DIWL 3, DIWL 4, and DIWL 5 did not exhibit biofilm formation on PET surfaces. However, the biofilm production by DIWL 1 and DIWL 2 is classified as weak biofilm production (Table 1).

It is proposed that a higher biodegradation potential was observed in bacterial biofilms. Due to the hydrophobic nature of the PET surface, bacterial adhesion requires the bacteria to possess a similar hydrophobic property (Chauhan *et al*., 2018). DIWL 1 and DIWL 2 exhibited biofilm, yet it is a weak formation onto PET. Meanwhile, DIWL 3, DIWL 4, and DIWL 5 showed no biofilm formation after incubation onto PET film for 24 hr. This may be due to the rigid structure of PET which causes the movement of bacteria on the PET surface to be limited thus causing biofilms to form on PET low or none (Demirkan *et al*., 2020). Webb (2012) also implies that the adhesion of bacteria to the surface of PET is limited. In this case, the formation of biofilm on PET can be induced by increasing the contact time of isolates with the PET or induced and improved by changing the structure of PET for instance-weathering process towards PET prior treated with bacterial isolates. The formation of biofilm is also influenced by the type of polymer. Demirkan *et al*. (2020) have reported that *B. cereus* ET30 and *B. subtilis* ET18 showed no biofilm formation occurred on the PET surface however, form biofilm on the surface of nylon.

Table 1. Crystal violet staining of biofilm formation absorbance (OD) onto PET surface at 595 nm and the classification of biofilm towards each bacterial isolate

	DIWL 1	DIWL 2	DIWL 3	DIWL 4	DIWL 5	Negative Control
Average OD	0.261	0.258	0.126	0.138	0.134	0.072
SD	0.0590	0.0254	0.025	0.033	0.042	0.014
ODc	-	-	-	-	-	0.114
Final OD	0.147	0.144	0.012	0.024	0.020	-
Classification	Poor	Poor	No biofilm production	No biofilm production	No biofilm production	-

Biodegradation of microplastic PET

The degradation and uptake of microplastics by various microbial species and consortia have been the focus of numerous studies due to their environmental significance. The ability of microorganisms to decompose plastic can be seen through the decrease in the weight of the plastic mass after the incubation period (Auta *et al*., 2017). The biodegradability potential of the bacterial isolates was further assessed by measuring the weight of PET after inoculated with DIWL 1, DIWL 2, DIWL 3, DIWL 4, and DIWL 5 for 20 days. The findings of this study showed the ability of bacterial isolation from PET plastic surfaces to degrade PET microplastics (Table 2). After 20 days of incubation with bacterial isolates, there was a decrease in the weight of PET mass treated with DIWL 1 and DIWL 2 recorded of 2.04% and 2.08% respectively, indicative of these isolates utilized PET as solely carbon source with the ability to form biofilm onto PET. When compared between DIWL 1 and DIWL 2 isolates, both isolates showed insignificant PET degradation activity (p>0.05). PET weight reduction has also been recorded when treated with *Nocardia* (Sharon & Sharon 2012). *Nocardia*-treated PET showed a weight loss of 0.2% over 20 days of incubation. If a comparison is made with this study, DIWL 1 (2.04%) and DIWL 2 (2.08%) exhibited greater biodegradation than *Nocardia* with a PET reduction of 10 times higher than PET treated *Nocardia* at the same incubation time. A study by Ruslan *et al*. (2018) found

that the weight of PET film after incubation with *Bacillus* sp. ITP 10.2.1 showed similar results as DIWL 1 and DIWL 2 (0.001 g) if the comparison was only focused on PET weight reduction. DIWL 1 and DIWL 2 exhibited higher degradation of PET plastic in comparison to PET-related bacterial isolate, *Streptomyces* species. A recent study by Belabbas *et al*. (2025) reported that *Streptomyces coeruleorubidus* SALG 1 achieved PET degradation rates of 0.124% and 0.049% over 60 days in the presence and absence of Tween 80, respectively.

DIWL 1 and DIWL 2 showed similar uptake rates of microplastic which are 0.0010 and 0.0011 $day^{-1,}$ respectively. When compared to other microbial species, DIWL 1 and DIWL 2 demonstrate a higher rate of PET uptake per day. For instance, the uptake rate of *B. gottheilii*, as reported by Auta *et al*. (2017), was 0.0008 day^{-1}. This is significantly lower than the rates observed for DIWL 1 and DIWL 2. Similarly, *Bacillus* sp. AIIW2 showed an even lower uptake rate of 0.000215 $days^{-1}$, according to Kumari *et al*. (2021). These comparisons highlight the superior efficiency of DIWL 1 and DIWL 2 in assimilating microplastic PET compared to these specific strains. Similarly, another study showed microbial consortia (*B. cereus* SEHD031MH and *Agromyces mediolanus* PNP3) utilize PET with an uptake rate of 0.0011 ± 0.0004 day^{-1} (Torena *et al*., 2021). Interestingly, *B. cereus* isolated from mangrove sediment has been shown to have an even higher uptake rate of PET compared to DIWL 1 and DIWL 2. A study by Auta *et al*. (2017) reported that *B. cereus* utilized PET at a rate of 0.0017 $days^{-1}$. This higher uptake rate suggests that isolated *B. cereus* may possess unique metabolic pathways or enzymatic activities that enable it to break down PET more rapidly than DIWL 1 and DIWL 2. *Brucella intermedia* IITR130, isolated from the lake sample, exhibited a PET film uptake rate of 0.0051 K day^{-1} - approximately four times higher than that of DIWL 1 and DIWL 2. This enhanced degradation is likely attributed to its ability to form biofilms on the PET surface, as observed in the SEM images (Srivastava *et al*., 2024).

Our study also evaluated the biodegradation of PET inoculated with DIWL 3, DIWL 4, and DIWL 5 were negligible via reduction of PET mass (Table 2). This is highly likely due to the incubation period of the polymer with the bacteria and the condition of the growth medium (Nowak *et al*., 2011). Degradation of the polymer will lead to a decrease in molecular weight caused by the cutting of polymer bonds and will subsequently produce oligomers. The resulting oligomers will then diffuse out of the polymer granules and cause a decrease in the mass weight of the polymer (Mallakpour & Nouruzi 2018). We deduce that isolates DIWL 3, DIWL 4 and DIWL 5 require a relatively long period to degrade PET as little or no mass weight loss of the polymer can be observed in the initial phase of degradation (Chamas *et al*., 2020). PET inoculated with DIWL 3, DIWL 4, and DIWL 5 exhibited insignificant weight loss probably due to the incubation period being too short to measure the weight loss of PET mass, and PET oligomer molecules may not have permeated out of PET granules even after the incubation period has ended.

Table 2. Analysis of PET mass reduction after 20 days incubation, percentage of PET reduction and removal constant (K) day^{-1}

Isolates	Initial weight average (g)	Final weight average (g)	Weight difference (g)	Percentage of weight loss (%)	Removal constant (K) (day^{-1})
DIWL 1	0.049	0.048	0.001	2.04	0.0010
DIWL 2	0.048	0.047	0.001	2.08	0.0011
DIWL 3	0.048	0.048	-	-	-
DIWL 4	0.049	0.049	-	-	-
DIWL 5	0.048	0.048	-	-	-

Analysis of PET using FTIR and SEM for detection of surface modification

Considering that attachment and subsequent deterioration are more feasible at the surface than within the bulk of the material, the surface-sensitive approach FTIR spectroscopy was utilized to trace the chemical alterations in degraded plastics (Nakkabi *et al*., 2015). Changes in the chemical structure of PET have been observed through the formation and loss of emission peaks for each functional group present in PET indicative microbial activity and is considered an important point in explaining the mechanism of plastic degradation by microbes (Auta *et al*., 2017; Torena *et al*., 2021). A high emission value indicates the presence of low molecular chains in the polymer while a low emission value indicates the presence of more molecular chains in the polymer (Habib *et al*., 2020).

The current study observed that DIWL 1, DIWL 2, DIWL 3, DIWL 4, and DIWL 5 are capable of utilizing PET proven by the changes in the molecular structure and chemical bonds on the polymer surface after the incubation period. Therefore, although the mass weight of PET microplastics treated with isolates DIWL 3, DIWL 4, and DIWL 5 showed no reduction, the changes in the chemical structure of PET could be evidence that PET microplastics were decomposed by bacterial isolates. PET inoculated with respective bacterial isolates exhibited a substantial increase in their aliphatic index (2925 cm^{-1}, 2871 $cm^{-1,}$ & 2869 cm^{-1}) (Figure 3) in line with the study performed by Roberts *et al*. (2020) on the biodegradation of PET inoculated with strain 10 (*Pseudomonas* sp. strain SWI36).

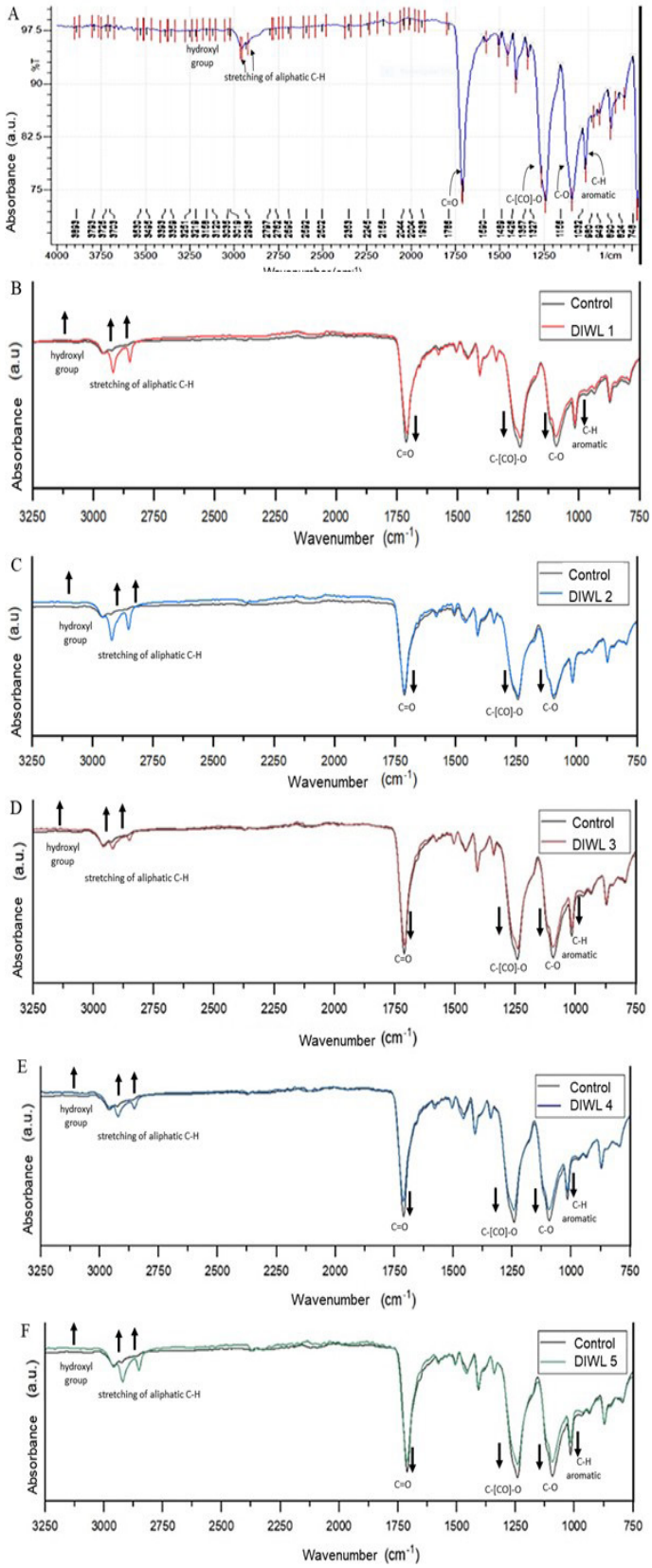

Fig. 3. Spectrum of PET after 20 days incubation. A) untreated PET, B) DIWL 1-treated PET, C) DIWL 2-treated PET, D) DIWL 3-treated PET, E) DIWL 4-treated PET and F) DIWL 5-treated PET.

As C=O (1740 cm^{-1}) and C-O (1248 cm^{-1}) bonds are broken during ester cleavage, carbonyl and ester functional group peaks decrease suggesting hydrolysation of former functional group to smaller molecular fragments was occurring and anticipated during PET degradation (Nakkabi *et al*., 2015; Roberts *et al*., 2020). The decomposition of PET produces chains in the form of

monomers, namely ethylene glycol and terephthalic acid. Due to the cutting of the ester bond on PET, it causes ethylene glycol to increase, and its concentration becomes saturated. This eventually causes a simultaneous increase in C-H bonds (Roberts *et al*., 2020) as observed in this study. This appears that bacterial isolates' attachment to PET changed the value of the functional group resulting monomer bond going through B-oxidation, the TCA cycle, and hence causing bacteria to utilize this carbon source for growth (Vague *et al*., 2019).

As for the physical alteration of the PET surface after 20 days of incubation (Figure 4), untreated PET remained smooth and unchanged surface morphology. Meanwhile, PET inoculated with isolates DIWL 1, DIWL 2, DIWL 3, DIWL 4, and DIWL 5 showed apparent differences compared to untreated PET. PET-treated isolates possessed erosions, cracks, grooves, and rough surfaces compared to untreated PET. There is also production of holes by DIWL 2 isolate towards the PET surface was also observed (Figure 4c).

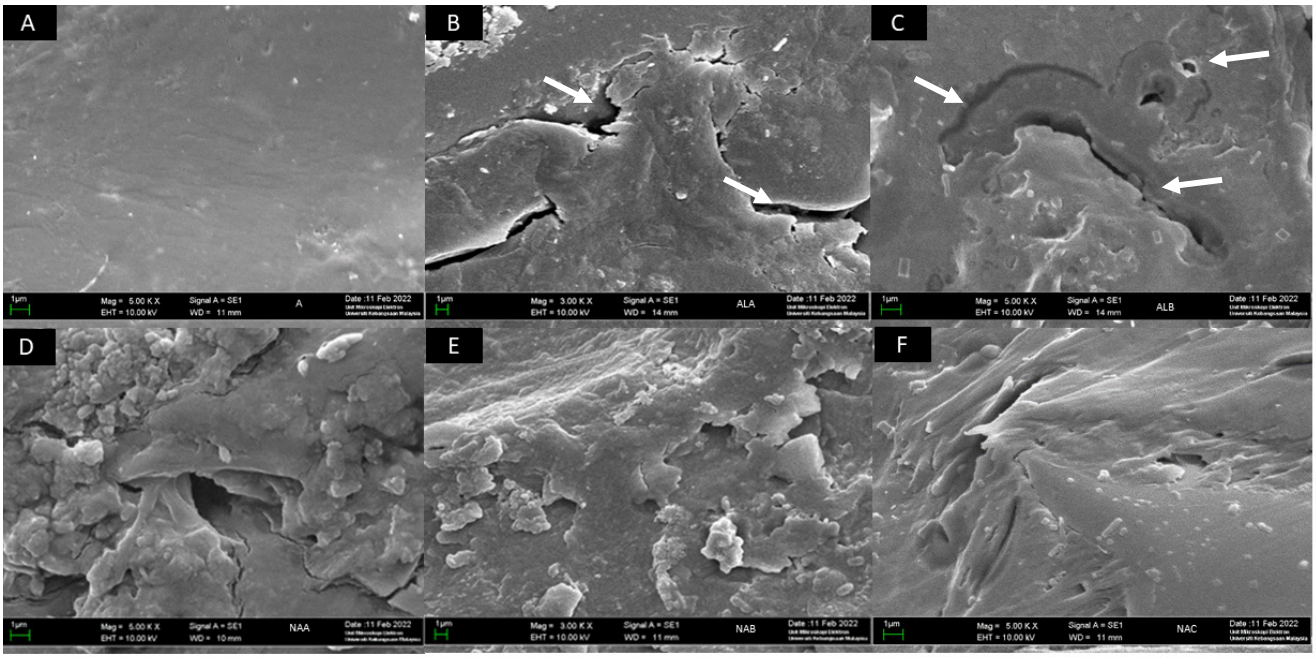

Fig. 4. SEM images show surface modification of PET granules. LCFBM containing PET granules with DIWL 1, DIWL 2, DIWL 3, DIWL 4, or DIWL 5 respectively were grown at 30°C for 20 days. After 20-day incubation, all PET granules were treated with 2% SDS to remove completely adherent bacteria onto the surface of PET before SEM. A) PET granules without inoculation of bacteria. The surface of PET is smooth. B) PET granules treated with DIWL 1 showed roughness and cracks (arrow). C) PET granules treated with DIWL 2 showed groove, roughness, and holes (arrow) D-F) PET granules treated with DIWL 3, DIWL 4 or DIWL 5 respectively showed roughness. Bars, 1µm (A to F).

Bacteria attached to the surface of PET will secrete surface-modifying enzymes such as lipase, cutinase, carboxylase, and protease and will subsequently change the surface of PET through a hydrolysis reaction (Mohanan *et al*., 2020). Changes in the physical structure of polymers, for example, the formation of holes and cracks are among the observations made to evaluate the biodegradation of polymers by bacteria (Raddadi & Fava 2019). Observation of PET treated with DIWL 1, DIWL 2, DIWL 3, DIWL 4, and DIWL 5 revealed esterase modifies and changes the morphology of the PET surface. Auta *et al*. (2017) also observed PET surface hardness and the formation of holes, cracks, and different grooves after 40 days of incubation with *B. cereus* and *B. gottheilii.*

Identification of isolated bacteria

Bacterial isolates that showed the ability to produce esterase were identified by 16S rRNA sequence. The isolated species belonged to four genera of class Bacilli (*Bacillus*). Deposition of a sequence of isolates DIWL 1, DIWL 3, and DIWL 4 into the NCBI database showed these isolates are *B. paramycoides* (Accession number: PP669688.1), *B. safensis* (Accession number: PP669643.1), and *B. luciferensis* (re-identified as *Gottfriedia luciferensis*; Accession number: PP669663.1) with percentage similarity of 97.96%, 98.51%, and 99.07%, respectively. Meanwhile, DIWL 2 (Accession number: PP669693.1) and DIWL 5 (Accession number: PP669666.1) showed a similar percentage of *B. cereus* with 98.33% and 99.14% respectively. The isolated microbes reflect the dominant bacteria community present in landfills and colonize plastic surfaces (Nowak *et al*., 2011; Puglisi *et al*., 2019).

CONCLUSION

This study reveals the potential of bacterial strains isolated from PET waste surfaces in landfills to degrade PET microplastics. The 20-day treated PET microplastic with *Bacillus* species (*B. paramycoides* DIWL 1, *B. cereus* DIWL 2, *B. safensis* DIWL 3, *B. luciferensis* DIWL 4, and *B. cereus* DIWL 5) has prevailed the modification of PET surface such as cracks and increase in roughness through observation with SEM. As for *B. paramycoides* DIWL 1 and *B. cereus* DIWL 2, the current study demonstrates PET degradation, with both bacterial isolates showing a degradation percentage of approximately 2%. All bacterial isolates demonstrated the alteration of PET's chemical structure, evidenced by an increase in aliphatic C-H functional groups, indicating

PET degradation. The findings suggest that *Bacillus*-associated PET surfaces facilitate PET decomposition and could potentially be utilized to mitigate PET waste.

ACKNOWLEDGEMENTS
This study was supported by the Ministry of Higher Education through the Fundamental Research Grant Scheme FRGS/1/2020/STG01/UKM/02/7.

ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.J. & Bhubalan, K. 2021. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. Progress in Earth and Planetary Science, 8: 12. https://doi.org/10.1186/s40645-020-00405-4

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

Belabbas, H., Djinni, I., Djoudi, W., Reti, W., Hamma, A., Souagui, S., Haddad, S. & Kecha, M. 2025. *Streptomyces coeruleorubidus* strain SALG1 derived seashore plastic bottle for the biodegradation of untreated plastic polymers. Environmental Science and Pollution Research, 1-18. https://doi.org/10.1007/s11356-025-36027-w

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M., Scott, S.L. & Suh, S. 2020. Degradation rates of plastics in the environment. ACS Sustainable Chemistry & Engineering, 8(9): 3494-3511. https://doi.org/10.1021/acssuschemeng.9b06635

Charnock, C. 2021. Norwegian soils and waters contain mesophilic, plastic-degrading bacteria. Microorganisms, 9(1): 1-18. https://doi.org/10.3390/microorganisms9010094

Chauhan, D., Agrawal, G., Deshmukh, S., Roy, S.S. & Priyadarshini, R. 2018. Biofilm formation by Exiguobacterium sp. DR11 and DR14 alter polystyrene surface properties and initiate biodegradation. RSC Advances, 8: 37590-37599. https://doi.org/10.1039/C8RA06448B

Chen, H.L., Nath, T.K., Chong, S., Foo, V., Gibbins, C. & Lechner, A.M. 2021. The plastic waste problem in Malaysia: management, recycling and disposal of local and global plastic waste. SN Applied Sciences 3(4): 1-15. https://doi.org/10.1007/s42452-021-04234-y

Dąbrowska, G.B., Janczak, K. & Richert, A. 2021. Combined use of Bacillus strains and Miscanthus for accelerating biodegradation of poly(lactic acid) and poly(ethylene terephthalate). PeerJ, 9: 1-21. https://doi.org/10.7717/peerj.10957

Demirkan, E., Guler, B.E. & Sevgi, T. 2020. Analysis by scanning electron microscopy of polyethylene terephthalate and nylon biodegradation abilities of Bacillus sp. strains isolated from soil. Journal of Biological & Environmental Sciences, 14(42): 107-114.

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

Habib, S., Iruthayam, A., Shukor, M.Y.A., Alias, S.A., Smykla, J. & Yasid, N.A. 2020. Biodeterioration of untreated polypropylene microplastic particles by Antarctic bacteria. Polymers, 12(11): 1-12. https://doi.org/10.3390/polym12112616

Issac, M.N. & Kandasubramanian, B. 2021. Effect of microplastics in water and aquatic systems. Environmental Science and Pollution Research 28(16): 19544-19562. https://doi.org/10.1007/s11356-021-13184-2

Kirmusaoğlu, S. 2019. The methods for detection of biofilm and screening antibiofilm activity of agents. In: Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods. S. Kirmusaoğlu (Ed.). IntechOpen, Rijeka. pp. 1-17. https://doi.org/10.5772/intechopen.84411

Kumari, A., Bano, N., Bag, S.K., Chaudhary, D.R. & Jha, B. 2021. Transcriptome-guided insights into plastic degradation by the marine bacterium. Frontiers in Microbiology, 12: 1-13. https://doi.org/10.3389/fmicb.2021.751571

Maity, S., Banerjee, S., Biswas, C., Guchhait, R., Chatterjee, A. & Pramanick, K. 2021. Functional interplay between plastic polymers and microbes: a comprehensive review. Biodegradation, 32(5): 487-510. https://doi.org/10.1007/s10532-021-09954-x

Mallakpour, S. & Nouruzi, N. 2018. Polycaprolactone/metal oxide nanocomposites: An overview of recent progress and applications. In: Biodegradable and Biocompatible Polymer Composites: Processing, Properties and Applications. N. G. Shimpi (Ed.). Woodhead Publishing, Cambridge. pp. 223-263. https://doi.org/10.1016/B978-0-08-100970-3.00008-0

Mohanan, N., Montazer, Z., Sharma, P.K. & Levin, D.B. 2020. Microbial and enzymatic degradation of synthetic plastics. Frontiers in Microbiology, 11: 1-22. https://doi.org/10.3389/fmicb.2020.580709

Molitor, R., Bollinger, A., Kubicki, S., Loeschcke, A., Jaeger, K.E. & Thies, S. 2019. Agar plate-based screening methods for the identification of polyester hydrolysis by *Pseudomonas* species. Microbial Biotechnology, 13(1): 274-284. https://doi.org/10.1111/1751-7915.13418

Nakkabi, A., Elmoualij, N., Saad, I.K. & Fahim, M.F. 2015. Biodegradation of poly(ethylene terephthalate) by *Bacillus subtilis*. International Journal of Recent Advances in Multidisciplinary Research, 2(12): 1060-1062.

Noik, V.J. and Tuah, P.M. 2015. A first survey on the abundance of plastics fragments and particles on two sandy beaches

in Kuching, Sarawak, Malaysia. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing, United Kingdom, 78: 012035. https://doi.org/10.1088/1757-899X/78/1/012035

Nowak, B., Pająk, J., Drozd-Bratkowicz, M. & Rymarz, G. 2011. Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. International Biodeterioration & Biodegradation, 65(6): 757-767. https://doi.org/10.1016/j.ibiod.2011.04.007

Puglisi, E., Romaniello, F., Galletti, S., Boccaleri, E., Frache, A. & Cocconcelli, P.S. 2019. Selective bacterial colonization processes on polyethylene waste samples in an abandoned landfill site. Scientific Reports, 9(1): 1-13. https://doi.org/10.1038/s41598-019-50740-w

Pui, C.F., Wong, W.C., Chai, L.C., Lee, H.Y., Noorlis, A., Zainazor, T.C.T., Tang, J.Y.H., Ghazali, F.M., Cheah, Y.K., Nakaguchi, Y., Nishibuchi, M. & Radu, S. 2011. Multiplex PCR for the concurrent detection and differentiation of *Salmonella* spp., *Salmonella typhi* and *Salmonella typhimurium*. Tropical Medicine and Health, 39(1): 9-15. https://doi.org/10.2149/tmh.2010-20

Qi, X., Yan, W., Cao, Z., Ding, M. & Yuan, Y. 2022. Current advances in the biodegradation and bioconversion of polyethylene terephthalate. Microorganisms, 10(1): 1-25. https://doi.org/10.3390/microorganisms10010039

Raddadi, N. & Fava, F. 2019. Biodegradation of oil-based plastics in the environment: Existing knowledge and needs of research and innovation. Science of the Total Environment, 679: 148-158. https://doi.org/10.1016/j.scitotenv.2019.04.419

Roberts, C., Edwards, S., Vague, M., León-Zayas, R., Scheffer, H., Chan, G., Swartz, N.A. & Mellies, J.L. 2020. Environmental consortium containing *Pseudomonas* and *Bacillus* species synergistically degrades polyethylene terephthalate plastic. Applied and Environmental Science, 5(6). https://doi.org/10.1128/mSphere.01151-20

Ruslan, R., Pekey, A.Y., Iqbal, M., Dewi, A.P. & Djamaan, A. 2018. Characterization of *Bacillus* sp. Itp 10.2.1 as degrading-bacteria of polyethylene terephthalate (PET) synthetic plastic. International Research Journal of Pharmacy, 9(11): 56-59. https://doi.org/10.7897/2230-8407.0911258

Sharon, M. & Sharon, C. 2012. Studies on biodegradation of polyethylene terephthalate: A synthetic polymer. Scholars Research Library, 2(2): 248-257.

Srivastava, P., Saji, J. & Manickam, N. 2024. Biodegradation of polyethylene terephthalate (PET) by Brucella intermedia IITR130 and its proposed metabolic pathway. Biodegradation. https://doi.org/10.1007/s10532-024-10070-9

Taniguchi, I., Yoshida, S., Hiraga, K., Miyamoto, K., Kimura, Y. & Oda, K. 2019. Biodegradation of PET: Current status and application aspects. ACS Catalysis, 9: 4089-4105. https://doi.org/10.1021/acscatal.8b05171

Torena, P., Alvarez-Cuenca, M. & Reza, M. 2021. Biodegradation of polyethylene terephthalate microplastics by bacterial communities from activated sludge. Canadian Journal of Chemical Engineering, 1-14. https://doi.org/10.1002/cjce.24015

Vague, M., Chan, G., Roberts, C., Swartz, N.A. & Mellies, J.L. 2019. *Pseudomonas* isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic. Sustainability, 11(1): 1-14. https://doi.org/10.1101/647321

Wang, P., Dai, H., Sun, B., Che, C. & Zhu, R. 2022. Bacteria community vertical distribution and its response characteristics to waste degradation degree in a closed landfill. Applied Sciences, 12(6): 2965. https://doi.org/10.3390/app12062965

Webb, H. 2012. Biodegradation of poly(ethylene terephthalate) by marine bacteria, and strategies for its enhancement (PhD). Swinburne University of Technology.

Webb, H.K., Arnott, J., Crawford, R.J. & Ivanova, E.P. 2013. Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate). Polymers, 5: 1-18. https://doi.org/10.3390/polym5010001

Xuyang, W., Khalil, K., Zhuo, T., Keyan, C., Nazir, M.Y.M., Aqma, W.S. & Hong, N.Q. 2025. Addressing the plastisphere: Sustainable approaches to combat plastic pollution. Science of the Total Environment, 959: 178105. https://doi.org/10.1016/j.scitotenv.2024.178105

Yang, J., Yang, Y., Wu, W., Zhao, J. & Jiang, L. 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environmental Science and Technology, 48: 13776−13784. https://doi.org/10.1021/es504038a

Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y. & Yu, F. 2020. Microbial degradation and other environmental aspects of microplastics/plastics. Science of the Total Environment, 715: 136968. https://doi.org/10.1016/j.scitotenv.2020.136968