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Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation☆

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ABSTRACT

The continuous accumulation of microplastics in the environment poses ecological threats and has been an increasing problem worldwide. In this study, eight bacterial strains were isolated from mangrove sediment in Peninsular Malaysia to mitigate the environmental impact of microplastics and develop a clean-up option. The bacterial isolates were screened for their potential to degrade UV-treated microplastics from polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS). Only two isolates, namely, *Bacillus cereus* and *Bacillus gottheilii*, grew on a synthetic medium containing different microplastic polymers as the sole carbon source. A shake flask experiment was carried out to further evaluate the biodegradability potential of the isolates. Degradation was monitored by recording the weight loss of microplastics and the growth pattern of the isolates in the mineral medium. The biodegradation extent was validated by assessment of the morphological and structural changes through scanning electron microscopy and Fourier transform infrared spectroscopy analyses. The calculated weight loss percentages of the microplastic particles by *B. cereus* after 40 days were 1.6%, 6.6%, and 7.4% for PE, PET, and PS, respectively. *B. gottheilii* recorded weight loss percentages of 6.2%, 3.0%, 3.6%, and 5.8% for PE, PET, PP, and PS, respectively. The designated isolates degraded the microplastic material and exhibited potential for remediation of microplastic-contaminated environment. Biodegradation tests must be conducted to characterize the varied responses of microbes toward pollutants, such as microplastics. Hence, a novel approach for biodegradation of microplastics must be developed to help mitigate the environmental impact of plastics and microplastic polymers.

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1. Introduction

More than 4.8 million tons of plastic wastes from land are deposited into the ocean (Boucher et al., 2016). In particular, microplastics (<5 mm in diameter) are widespread in the global marine environment (Cozar et al., 2014; Eriksen et al., 2014) and an increasing source of anthropogenic litter in aquatic environments (Bakir et al., 2014). Microplastics make up 92.4% of plastic waste (Santana et al., 2016) and consist mainly of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride, nylons, polylactic acid, polyamide, and polyethylene terephthalate (PET) (Carr et al., 2016). Although microplastics are resistant to degradation and persistent in the environment, they can be degraded by some microbes (Paco et al., 2017).

Microplastics are distributed globally in the world's oceans in water columns, surface waters, along shorelines, and at bottom sediments (Van Cauwenberghe et al., 2015). These wastes contaminate rivers, lakes, and ponds (Wagner et al., 2014; Dris et al., 2015; Eerkes Medranos et al., 2015). Microplastics originate from different sources; primary microplastics are intentionally produced in microscopic scale and used in cosmetics, toothpaste, exfoliating scrubs, hand cleaners, clothing, and drilling fluids (Duis and Coors, 2016). Secondary microplastics originate from the weathering of macroplastic debris (Ballent et al., 2016). In general, microplastics enter the ocean through several marine- and terrestrial-based activities. Microbeads in toothpaste and other cosmetic products enter the aquatic environment through wastewater treatment plants and drainage systems (McCormick et al.,

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2016; Murphy et al., 2016). The degradation of large plastic debris from waste dumps or landfills can also serve as source of microplastics to oceans (Alomar et al., 2016). When large plastic particles fragment into small particles, the abundance and encounter rate of microplastics with marine biota both increase.

Microplastics are consumed by a wide-range of marine organisms, such as filter organisms, invertebrates, fish, mammals, and birds, and can potentially interfere with the food chain. Batel et al. (2016) reported the transfer of microplastics and associated toxic substances from the brine shrimp *Artemia* sp. nauplii to zebra fish that fed on the nauplii. Microplastic ingestion poses risks to marine organisms by causing false satiation, pathological stress, reproductive complication (Green, 2016), reduced growth rate (Lonnstedt and Eklov, 2016), oxidative stress, liver inflammation, and lipid accumulation in the liver (Lu et al., 2016). This phenomenon may eventually lead to granulocytoma formation, lysosomal membrane destabilization, increased metabolic stress, blocked enzyme production, and low steroid hormone level (Fossi et al., 2016; Sutton et al., 2016). Microplastics adsorb and accumulate metals and persistent organic pollutants from the surrounding environment, thereby serving as vectors for heavy metal contamination in the marine environment (Brennecke et al., 2016). These chemicals can leach into animal tissues or other pristine environments and can cause endocrine disruption, mortality, delayed ovulation, and hepatic stress (Ogunola and Palanisami, 2016).

Microorganisms are opportunistic and possess an inherent ability to adapt to almost every environment (Brooks et al., 2011; Aujoulat et al., 2012). Microorganisms also exhibit potential to transform a variety of compounds, including plastic polymers. This adaptive feature aids microbes to metabolize significantly in the presence of pollutants and, in some cases, enhance degradation and biotransformation (Luigi et al., 2007). For example, studies indicated the viability of bacterial isolates for the remediation of environmental pollutants, including heavy metals (Emenike et al., 2016), lubricating oil (Abioye et al., 2010), crude oil (Auta et al., 2014), benzo[*a*]pyrene (Aziz et al., 2017), and polycyclic aromatic hydrocarbons (Mohd Radzi et al., 2015).

Numerous scientific studies have examined the distribution, ingestion, fate, behavior, quantification, and effect of microplastics (GESAMP, 2015). However, to date, methods for microplastic cleanup and/or remediation remain inconclusive. These methods include biological degradation and utilization of plastic polymers. Yoshida et al. (2016) investigated the degradation of PET by the bacterium *Ideonella sakaiensis* 201-F6, which can use PET as a sole energy and carbon source for growth. Mohan et al. (2016) reported the potential of *Pseudomonas* sp. and *Bacillus* sp. to degrade brominated high-impact PS. Paco et al. (2017) evaluated the response of the fungus *Zalerion maritimum* to different incubation times of PE pellets. Results demonstrated that the fungus can utilize PE under the tested conditions and decreased the mass and size of the pellets. These findings indicated the potential of naturally occurring fungi to degrade microplastics. Sowmya et al. (2014) described the degradation of PE by *Bacillus cereus*. Harshvardhan and Jha (2013) indicated the degradation of PE by marine bacteria (*Kocuria palustris* M16, *Bacillus pumilus* M27, and *Bacillus subtilis* H1584); these bacterial species exhibited weight loss of 1%, 1.5%, and 1.75% after 30 days of incubation, respectively. Other polymer-degrading bacteria include *Pseudomonas stutzeri*, *Alcaligenes faecalis*, *Pseudomonas putida*, *Brevibacillus borstelensis*, *Streptomyces* sp., and *Staphylococcus* sp. (Ghosh et al., 2013; Caruso, 2015).

During polymer degradation, the microbes first adhere onto the polymer surface, thereby exposing itself to microbial colonization. Polymer colonization is followed by the secretion of extracellular enzymes, which bind to the polymer and cause hydrolytic cleavage (Lucas et al., 2008; Shah et al., 2008). The polymer is subsequently degraded into low-weight polymers and mineralized to carbon dioxide (CO_2) and water (H_2O), which are used by the microbe as energy source (Tokiwa et al., 2009). Microplastic particles in the organism pass through the cellular membrane, where they are broken down within the cells of the organism by cellular enzymes (Gewert et al., 2015).

Using microbes to degrade microplastics will enhance biodegradation without causing any harm to the environment (Bhardwaj et al., 2012). Therefore, identifying microbes that can degrade microplastics is a promising and environmentally safe strategy to facilitate natural bioremediation and influence the cleaning of natural ecosystems without imposing adverse impacts. Mangrove forests possess significant microbial diversity (Kathiresan, 2003; Thatoi et al., 2012), which plays significant roles in various environmental processes and applications (Sahoo and Dhal, 2009). High temperature, salinity, pH, and organic matter content and low aeration and moisture levels improve the substrate conditions to be conducive for the development of microbial populations (Ghizelini et al., 2012). In addition, coastal mangroves were traditionally favored as dumping sites for solid waste disposal (Kathiresan and Bingham, 2001). Given that most wastes (mostly made of plastics) undergo degradation/biochemical transformations despite the salinity and moisture level of the environment, potential degraders may inhabit such environments.

This study aimed to provide remediation solution to microplastic-polluted environment by using bacterial isolates from mangroves in Peninsular Malaysia. This work also evaluated the potential of marine bacteria isolated from the mangrove environments for degradation of microplastics.

2. Materials and methods

2.1. Polymer materials

Chemicals from Sigma Aldrich Chemical Co. (USA) included PE powder (white) with 75 μm particle size and density of 0.94 g/mL at 25 °C, PP granules (white, spherical) with density of 0.9 g/mL at 25 °C, PS granules (white/spherical) with density of 1.59 g/mL at 25 °C, and PET granules (granular/milky white) with density of 1.68 g/mL at 25 °C. For the degradation experiments, microplastics were obtained by grating/cutting commercial plastic materials from plastic-producing industries by using a bastard-cut hand file and scissors; these materials were made of PE, PP, PET, and PS. The grated plastic obtained was passed through a 250 μm sieve (mesh no. 60, Chunggye Industrial Mfg. Co., Seoul, Republic of Korea) to screen large debris. Each plastic was irradiated for 25 days under UV light and stored for further use. The sizes of the prepared plastic debris were measured using an optical microscope (IX71, Olympus, Japan) equipped with 4 × lens (Olympus).

2.2. Sediment sample collection and characterization

Mangrove sites selected in this study served as a representative of east, west, south, and north of Peninsular Malaysia. The sediment samples were collected bimonthly for one year from Matang mangrove in Perak (4°50′25.80" N, 100°38′9.60" E), Cherating mangrove in Pahang (4°7′36.15" N, 103°23′29.46" E), Tanjung Piai in Johor (1°16′5.20" N, 103°30′31.36" E), Sekam mangrove in Melaka (1°37.84" N, 103°26′30.61" E), Sedili Besar in Johor (1°55′54.39" N,104°7′27.25" E), and Pasir Puteh mangrove in Kelantan (5°50'.79" N, 102°25′41.07" E) of Peninsular Malaysia. Samples were obtained at 1 cm intervals at 0–4 cm depth in the sediment from three different points with a quadrat of 0.5 m × 0.5 m placed 2 m apart from high tide in undisturbed areas (Nor and Obbard, 2014). The obtained samples were placed into sterile plastic bags and

transported to the laboratory for further analysis. Excavated samples were pooled accordingly and analyzed for pH, salinity, and temperature using a multiprobe meter (YSI Professional Plus, USA). The parameters are presented in Table 1. All assessments were carried out in triplicates. In each trial, triplicate samples were evaluated to obtain the discrete average value. Pooled values represent the mean sum of the average values on each trial time.

2.3. Bacterial isolation and identification

Bacterial species were isolated by mixing 1 g of sediment sample with 9 mL of normal saline water (0.9% NaCl). The mixture was vortexed for 3 h at 180 rpm using Lab-Line 3521 orbit shaker. The resulting suspension was serially diluted, plated on nutrient agar (NA), and incubated at 37 °C for 24 h (Emenike et al., 2016). Single colonies were further subcultured on freshly prepared NA to obtain distinct individual pure cultures suitable for identification. All experiments were carried out in triplicates. Isolated bacteria were identified using the Biolog GEN III microplate protocol. An Omnilog reader was used to identify the bacterial species contained in the Biolog's Microbial Identification Systems Software.

2.4. Screening of bacterial isolates for microplastic degradation

Mineral salt media (MSM) were used to screen for microplastic degradation using a method described by Kannahi and Sudha (2013) with slight modifications. The media contained all nutrients necessary for bacterial growth, except for a carbon source. Eight bacterial isolates were assayed for their ability to utilize PE, PS, PET, and PP polymers as sole source of carbon and energy for growth. Each individual isolate was grown in MSM infused with 0.5 g of specific plastic polymers and incubated for 4 weeks at room temperature. A control set was maintained (inoculation on media without polymer) simultaneously, and the media were observed for growth. All experiments were carried out in triplicates.

2.5. Microbial inoculum preparation and biodegradation experiments

Bacteria isolated and identified as microplastic-degrading microorganisms were grown on freshly prepared NA to obtain pure cultures at 33 °C for 24 h before inoculation in nutrient broth. These bacteria were also allowed to grow to a stationary phase in rotating shaker at 29 °C at 150 rpm. Individual suspensions at the same physiological phase, i.e., 1.09 absorbance (ABS) at 600 nm, were pooled in equal proportions to prepare inocula for biodegradation. Absorbance works on the principle of light passage; in this context, absorbance relates to increased cell density of the medium, which reflects growth. The cell densities of the inocula were adjusted to 3.8×10^8 colony-forming units (CFU) per mL for the biodegradability experiment.

Pure cultures of the plastic-degrading bacteria isolated (3.8×10^8 CFU/mL cells) were inoculated into 270 mL of MSM broth in flasks containing 0.5 g of UV-treated microplastics (PE, PP, PET, and PS). The flasks containing non-inoculated MSM supplemented with the polymer particles served as control. Hence, the control flask contained 300 mL of mineral salt broth and 0.5 g of the microplastics. Triplicates were maintained for all experiments. The flasks were left on a shaker (150 rpm). The optical density (OD), pH, and microbial count were monitored at every 10 days for a period of 40 days. Measured OD is a reflection of the growth response of microbes in the designed aqueous system. Each measurement and evaluation were carried out at each trial (three trials), and they included OD assessment for growth, acidity–alkalinity evaluation (pH), and the population load.

2.5.1. Determination of dry weight, reduction rate, and half-life of residual microplastic particles

After 40 days of incubation, the microplastic polymers were recovered from the broth through filtration. Plastic particles were washed with 70% ethanol and dried in hot air oven at 50 °C overnight. Residual polymer weight was determined to measure the extent of degradation (Mor and Sivan, 2008; Mohan et al., 2016). The initial weights of the preincubated microplastic samples were measured following the same technique mentioned above. The plastic polymer degradation was evaluated in terms of percentage weight loss using the following formula:

$$\text{Percentage weight loss} = \left(\frac{\text{Initial weight of polymer} - \text{Final weight of polymer}}{\text{Initial weight of polymer}}\right) \times 100. \quad (1)$$

Data were further processed to determine the rate constant of microplastic polymer reduction using the first-order kinetic model as follows:

$$K = -\frac{1}{t}\left(\ln\frac{W}{W_o}\right) \quad (2)$$

where K is the first-order rate constant for polymer uptake per day, t is the time in days, W is the weight of residual polymer (g), and W_O is the initial weight of polymer (g).

Following the generation of the microplastic polymer removal rate constant, the half-life ($t_{1/2}$) was calculated (Alaribe and Agamuthu, 2015) according to Eq. (3):

$$\left(t_{1/2}\right) = \ln(2)/K. \quad (3)$$

2.5.2. Fourier transform infrared (FTIR) analysis of microplastic polymers

Changes in the structure of all the microplastic polymers with subsequent bacterial incubation were analyzed by FTIR Spectroscopy (Perkin-Elmer 400 FT-IR/FT-FIR) in the frequency range of 4000–450 cm^{-1}. This analysis was carried out on all samples

Table 1
Means of environmental parameters for each mangrove site across months.

Sample site	Parameter		
	Temperature (°C)	pH	Salinity (ppt)
Matang mangrove (Perak)	28.9 ± 0.244	6.75 ± 0.104	9.56 ± 4.219
Sekam mangrove (Melaka)	28.8 ± 0.081	7.44 ± 0.463	29.51 ± 0.493
Tanjung Piai mangrove (Johor)	29.2 ± 0.368	5.99 ± 0.175	30.83 ± 2.369
Cherating mangrove (Pahang)	28.6 ± 0.163	6.32 ± 0.225	21.50 ± 0.848
Sedili Besar mangrove (Johor)	29.4 ± 0.355	7.50 ± 0.401	8.99 ± 1.966
Pasir Puteh mangrove (Kelantan)	28.8 ± 0.081	7.23 ± 0.388	19.39 ± 0.723

incubated with bacterial strains and on the uninoculated control.

2.5.3. Scanning electron microscope (SEM) analysis

The changes in surface morphology of the PE, PP, PET, and PS microplastics were investigated after the 40 days incubation period with *B. cereus* and *B. gottheilii* using field emission scanning microscope (Leica EM SCD005, Australia). The degraded microplastics with the controls were sputter coated with a gold layer at 25 mA under Ar atmosphere at 0.3 MPa and subsequently examined under the SEM (Sekhar et al., 2016).

2.5.3.1. Statistical analysis. Statistical analysis of data was carried out using ANOVA from SPSS software 21.0.

3. Results and discussion

3.1. Isolation and identification of bacteria

Eight bacterial strains were isolated from mangrove environments. Isolates were aerobic bacteria, and growth patterns were sufficiently distinct to enhance the identification and differentiation into individual isolates. The isolated species belonged to six genera of class Bacilli (*Bacillus*) and two genera class of γ-Proteobacteria (*Acinetobacter* and *Stenotrophomonas*). The isolated bacteria included strains of *B. cereus*, *Bacillus cibi*, *Acinetobacter schindleri*, *B. gottheilii*, *Bacillus pseudomycoides*, *Bacillus stratosphericus*, *Bacillus aquimaris*, and *Stenotrophomonas maltophilia*. The isolated microbes reflect the native bacteria community present in mangrove environments (Saimmai et al., 2012; Basak et al., 2016).

3.2. Primary screening of isolates for polymer degradation

Two bacterial isolates, namely, *B. cereus* and *B. gottheilii*, can grow on the MSM containing PE, PP, PET, and PS as sole carbon source. This result suggested that these two isolates possess enzymatic mechanism required to degrade PE, PET, PP, and PS. Their ability to utilize the polymers could have resulted from their adaptability to plastic-infested environment.

3.3. In vitro biodegradation assay: shake flask experiment

The biodegradability potential of the bacterial isolates was further assessed in an aqueous medium containing PE, PET, and PS microplastics for *B. cereus* and PE, PP, PET, and PS microplastics for *B. gottheilii* as the sole carbon source (Tables 2 and 3).

The potentials of the isolates to significantly affect the weight of the microplastics after 40 days were compared with the calculated percentage weight loss, rate of reduction constant, *K*, and half-life. After 40 days, the percentage weight loss of PE, PET, and PS by *B. cereus* was 1.6%, 6.6%, and 7.4%, respectively (F-value = 1.035; P = 0.411). The highest reduction rate (0.0019 day^{-1}) and half-life of approximately 363.16 days were observed in the degradation of PS by the isolate. The calculated microplastic removal rate constant (*K*) and the corresponding half-life further supported the degree of activities within the aqueous medium. Results depicted that 0.0019 g of PS microplastic was removed or taken up by *B. cereus* on a daily basis. Additionally, *B. cereus* will need approximately 363 days to reduce the PS microplastic polymer to its half (i.e., from 0.50 g to 0.25 g). No mass change was observed in control (uninoculated) microplastics.

B. gottheilii recorded a percentage weight loss of 6.6%, 3.0%, 3.6%, and 5.8% for PE, PET, PP, and PS microplastics, respectively (F-value = 0.476; P = 0.71). The uptake rate of the microplastics by *B. gottheilii* was in the range of 0.00076–0.0016 day^{-1}, with the highest uptake rate (0.0016 day^{-1}) and a half-life of 431.25 days recorded for the degradation of PE. The half-life of 907.89, 758.24, and 460 days were recorded for PET, PP, and PS, respectively. This removal rate might be from the genetic make-up of the isolate, which could discretely possess considerable polymer degradation capacity.

The changes in mass and molecular weight of the microplastics by the bacterial isolates could have resulted from bond cleavage caused by the pretreatment of the microplastics with UV radiation: exposure of plastic polymers to UV results in increased carbonyl and terminal double bond indices. The bacterial isolates possess functional groups that can attach to the microplastic surfaces (Lucas et al., 2008; Harshvardhan and Jha, 2013). On the 5th day of incubation, some of the microplastic particles sank to the bottom of the medium. The sinking could probably be attributed to the increased density due to biofouling as a result of colonization by the organisms (Andrady, 2011; Auta et al., 2017). At the end of the 40 days of incubation, the visual effect of the degradation process was observed in the yellowing of the microplastic polymers, which signified the initial phase of disintegration process and characterized the bacterial colonization on the polymer surface (Hemjinda et al., 2007). Harshvardhan and Jha (2013) recorded weight loss of 1%, 1.5%, and 1.75% for PE by *K. palustris*, *B. pumilus*, and *B. subtilis* isolated from pelagic water, respectively.

The growth profile of the bacterial isolates during the in vitro biodegradation assay showed that both isolates demonstrated

Table 2
Mass reduction efficiency and the growth kinetics of *B. cereus* in microplastic-infused mineral salt media.

Microplastic type	Percentage weight loss (%)	F-value	Significance	R^2	Removal constant (k) day^{-1}	Half-life (In 2/k) (days)
PE	1.6			0.8228	0.0004[a]	1725.00
PET	6.6	1.035	0.411	0.5851	0.0017[a]	405.88
PS	7.4			0.9192	0.0019[a]	363.16

Values followed with [a] indicate no significance at P < 0.05 level.

Table 3
Mass reduction efficiency, removal rate, and half-life of *B. gottheilii* in microplastic-infused MS media.

Microplastic type	Weight loss (%)	F-value	Significance	R^2	Removal constant (k) day^{-1}	Half-life (In 2/k) (days)
PE	6.2			0.8936	0.0016[a]	431.25
PET	3.0	0.476	0.707	0.6265	0.00076[a]	907.89
PP	3.6			0.9506	0.00091[a]	758.24
PS	5.8			0.9986	0.00147[a]	460.00

Values followed with letter [a] indicate no significance at P < 0.05 level.

varying metabolic responses to the different microplastics (Figs. 1 and 2).

The growth curve of *B. cereus* reflected varied growth patterns to different microplastics. The growth patterns were characterized by significant growth phase, indicating the increase in the microbial cell load. This phase was followed by mild, stable, and sharp decline phases. The growth response commonly showed similar growth pattern across polymer types when the microbe responded exponentially to the microplastics on the 10th day (PE = 1.13 ABS, PET = 1.08 ABS, and PS = 1.04 ABS compared with the initial 0.24 ABS) before showing decline. This result corresponded with increased cell counts on the same day (PE = 4.8×10^{11} CFU/mL, PET = 4.4×10^{11} CFU/mL, and PS = 4.9×10^{11} CFU/mL). However, the enhanced growth rate observed through the measured OD on the 10th day depicted lack of the optimal performance/response of *B. cereus* upon exposure to the microplastics. This result may imply the duration of the most favorable period of interaction between the designated microplastics and the bacterial cell that allows for rapid metabolism. Such observation can be attributed to that the overall response across the 40 days of exposure showed that the microbe, when exposed to PS, accelerated toward a positive growth pattern from 1.04 ABS to 1.10 ABS. On the contrary, the growth declined upon exposure to PE and PET. Therefore, *B. cereus* potentially exhibited more survival potential between the 10th and 20th days of exposure in the presence of PS than that with PE and PET microplastic polymers, as shown in the almost stationary phase demonstrated in the Figure by the organism. Furthermore, the PS tolerance potential of *B. cereus* was evident along the decline phase because its reduction in measured OD was not as steep as that in the exposure to PE and PET. This result may be attributed to the discrete potential of *B. cereus* to influence significantly the bonds of PS across time in order to maintain its metabolic activities, which include feeding and generation/doubling. Hence, the order of metabolic response of *B. cereus* to designated microplastics prioritized PS before PE and PET but did not vary statistically (F = 1.927; P = 0.226). Nevertheless, this order of response may require additional molecular assessment to evaluate the potentials of active binding sites on the microbe, which enhance selective attachment and metabolic interactions, whether at the functional group or associated derivatives during exposure. *B. cereus* showed similar potential in degrading the microplastics (PE, PET, and PS) despite the high uptake and reduction rate in PS, which could probably be due to the physiological state of the bacterial species. Moreover, the evaluation aimed to identify the potential effect of *B. cereus* on the microplastics in general. Nonetheless, the comparison on the degradation capacity for individual polymer was based on the discrete response of *B. cereus* upon exposure to the microplastics, which is in contrast to the overall hypothesis that degradation potential will be equal across polymers.

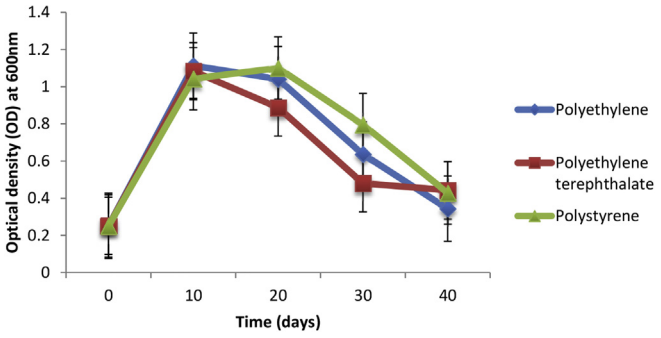

Fig. 1. Growth curve of *B. cereus* during biodegradation studies.

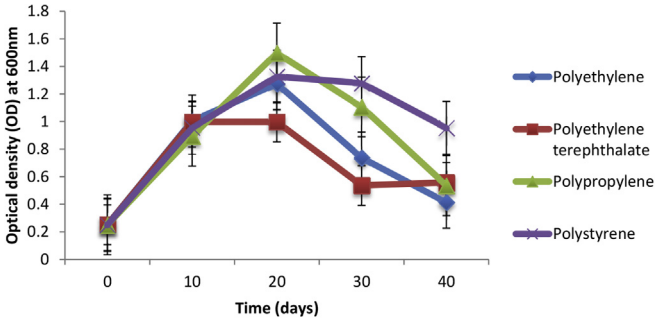

Fig. 2. Growth curve of *B. gottheilii* during biodegradation studies.

B. gottheilii demonstrated a sudden decrease in transmittance, that is, increase in absorbance, which depicted exponential growth. This trend can be observed from day 0 to day 20 for PE, PP, and PS, and it also coincided with the logarithmic increase in the number of bacterial cells during the same period (3.8×10^8–2.3×10^{11}, 3.8×10^8–2.9×10^{10}, and 3.8×10^8–2.7×10^{10} CFU/mL), with OD readings of 1.28, 1.49, and 1.33 ABS for PE, PP, and PS, respectively (Fig. 2). The highest OD was recorded in PP-induced media (1.49 ABS). However, for PET, a stationary growth phase was attained by the isolate on days 10–20 (0.99 ABS). This stationary growth phase could be due to the accumulation of waste materials, toxic metabolites, or shifts in the conditions of the medium, thereby creating an unfavorable environment for bacterial growth. The growth declined (0.99–0.54 ABS) on day 30. Afterward, the growth further increased from 0.54 ABS to 0.56 ABS on day 40. The effect of *B. gottheilii* on the microplastics revealed that the isolate statistically varied with responses to microplastic exposure (F-value = 4.806; P = 0.034). According to the results, the order of biodegradability of the microplastics by *B. gottheilii* was as follows: PE (6.2%) > PS (5.8%) > PP (3.6%) > PET (3.0%).

B. gottheilii showed higher capacity to degrade a wide variety of microplastics than that of *B. cereus*, indicating their degradability potential upon exposure to PP. The differences in response to the different microplastics could indicate the variations that exist in metabolic rate, polymer uptake mechanism, and associated genetic alteration within the individual isolates. This result implied that *B. gottheilii* is a broad-spectrum bacterium with affinity to all four microplastics (PE, PET, PP, and PS). This bacterium could also be a potential multiplastic degrader. When introduced into microplastic-contaminated environments, microbes can degrade microplastics of different polymers types and are thus important for environmental remediation of pollutants.

3.4. Fourier transform infrared (FTIR) analysis of microplastic polymers

The chemical structures of the biodegraded microplastics were analyzed through FTIR spectroscopy analysis (Perkin-Elmer 400 FT-IR/FT-FIR) within the frequency range of 4000–450 cm^{-1}. The analysis was performed to examine the changes in the chemical structures of the microplastics as a result of the action of the bacterial isolates on the polymers and confirm the biodegradation of the different microplastics. The FTIR spectra of the uninoculated (control) microplastics and different microplastic types incubated with *B. cereus* and *B. gottheilii* for 40 days in aqueous medium are shown in Supplementary Figs. S1–S11.

Fig. S1 shows the peak at 1798 cm^{-1} in the PE control experiment (uninoculated PE), which is assigned to C=O. The carbonyl band disappeared in the FTIR spectra of PE inoculated with *B. cereus*. New absorption bands appeared at 3738 and 3419 cm^{-1} in

both PE inoculated with *B. cereus* (Fig. S2) and PE inoculated with *B. gottheilii* (Fig. S3); these bands are attributed to N–H and O–H bonds, respectively, and could possibly be due to the formation of amino and hydroxylated compounds. The N–H band that appeared in PE inoculated with *B. cereus* split into two, which showed the presence of a primary amine. The formation of a new peak at 1460 cm^{-1} is attributed to C=C of an aromatic compound. This peak reflects the intrinsic constituents of *B. cereus*, which is chemically complex, especially in the protein level, and the amino and neutral polysaccharides (Ma et al., 2014; Matz et al., 1970). Hence, the replacement of the carbonyl band with amine bands indicated the favorable metabolism of the strain in microplastic-induced environment and possible evidence of gradual interference on the chemical structure of the PE, which could cause degradation. Furthermore, the elongation of the peak at 730 cm^{-1}, the disappearance of the peak at 848 cm^{-1}, and the phenolic peak of C–O at 1038 cm^{-1} are also observed in both PE inoculated with *B. cereus* and PE inoculated with *B. gottheilii*; these findings could be attributed to the oxidation of the PE by both isolates, resulting in decreased molecular weight of the polymer. Wilkes and Aristilde (2017) reported that the formation of biofilms on PE alters the polymer by oxidation reactions. Thus, the oxidation reaction that occurs could have increased the hydrophilicity of PE by producing functional groups, such as carbonyl, alcohol, phenol, and hydroxyl groups, which enhance the bacterial adherence and biodegradation.

In contrast to that in the uninoculated control PP sample (Fig. S4), the formation of a broad and strong peak at 3300 cm^{-1}, which is assigned to O–H, was observed on PP microplastic inoculated with *B. gottheilii* (Fig. S5). The carbonyl band (C=O) present at 1739 cm^{-1} in PP uninoculated control sample was strong and elongated, but it shifted to 1645 cm^{-1} in PP microplastic inoculated with *B. gottheilii*. Similarly, a strong C–O peak formed at 1014 cm^{-1} on PP microplastic inoculated with the isolate. The C–H aliphatic stretching peaks at 2950–2839 cm^{-1} and C–H aliphatic bending peaks at 1376 cm^{-1} also showed evident reduction. The reduction of the peak at 1455 cm^{-1} is assigned to C=C aromatic stretching. The elongation of the absorption peaks at 998 and 973 cm^{-1} is attributed to C=C bending.

The peak 1046 cm^{-1} of the phenolic C–O band in PET control sample (uninoculated PET) (Fig. S6) was absent on both PET microplastics inoculated with *B. cereus* (Fig. S7) and PET microplastics inoculated with *B. gottheilii* (Fig. S8). The PET control experiment showed its peak at 470 cm^{-1}. *B. cereus* induced a shift to 480 cm^{-1} on a different treatment, which expressed the action of *B. cereus* on PET microplastics.

Evident elongation and reduction were observed in almost all the peaks in both PS inoculated with *B. cereus* (Fig. S10) and *B. gottheilii* (Fig. S11) spectra compared with those of uninoculated PS control sample (Fig. S9). The elongations observed in PS inoculated with *B. cereus* included C–H (aromatic) peaks at 3082–3026 cm^{-1}, C–H (aliphatic) peaks at 2922–2850 cm^{-1}, and C=C (aromatic) peaks at 1601 and 1492–1452 cm^{-1}. The phenolic bands C–O at 1027 cm^{-1} and C–H (aliphatic bending) peaks at 1370 cm^{-1} and the reduced size of the peaks were observed in PS inoculated with *B. gottheilii.* The oxidation products formed at different frequencies indicated the degradation of the polymer by the microbes. Previous reports demonstrated that changes in functional groups (addition or disappearance of functional groups) and side-chain modifications are due to microbial activities (Harshvardhan and Jha, 2013; Sekhar et al., 2016).

The observed shift in peaks and the formation of oxidation products, such as carbonyls, hydroxyls, esters, aromatics, and alcohols, observed in the treated microplastic samples reflected the changes in the chemical structure of the microplastics. This change was due to the adherence of the microbial isolates, which altered the polymer through oxidation reactions. These new functional groups formed are metabolized in the bacterial cell through β-oxidation and tricarboxylic acid cycle, thereby enhancing biodegradation (Shah et al., 2008; Wilkes and Aristilde, 2017).

3.5. SEM analysis of biodegraded microplastics

SEM observation demonstrated that the microplastics showed morphological changes. After 40 days of incubation with the isolates, some of the microplastic surfaces became rough and possessed numerous holes/pores, erosions, cracks, and grooves [Fig. S12 (b), S12 (c), S13 (b), S14 (a), S14 (b), SI 5 (a), and S15 (b)]. The uninoculated samples (control) remained smooth and unchanged [Fig. S12 (a), S13 (a), S 14 (a), and S15 (a)]. This result provided evidence for the deterioration of the microplastics due to the action of the microbes and confirmed the degradation capacity of the microbes. Previous studies utilized SEM micrographs as analytical tool to demonstrate erosions, cavities, and pores formed on plastic films to indicate the extent of colonization and degradation (Sowmya et al., 2014). The evaluation of macroscopic modifications in plastic materials, such as roughening of the surface, formation of holes/pores, cracks, and changes in color, is a method used to estimate polymer biodegradation (Lucas et al., 2008; Rosa et al., 2004).

4. Conclusion

This study demonstrated the potential of bacteria isolated from mangrove sediments to degrade microplastics. The in vitro biodegradation study of the different microplastics suggested the suitability of two mangrove bacteria, namely, *B. cereus* and *B. gottheilii*. Growth patterns of the isolates in microplastic-infused media, formation of new functional groups, and reduction in the absorption characteristic peaks of the microplastics from FTIR analysis, weight loss, and subsequent morphological changes observed in SEM images confirmed the process of biodegradation and the ability of the isolates to utilize microplastics. Therefore, the strategy demonstrated in the study will potentially play significant role toward the identification of suitable microplastic degraders. This strategy is considerably important because the selection of the most suitable degradation strategy requires the optimization of capable microbes over specific pollutant. Hence, when properly optimized and applied on polluted sites, the degradation effect will reduce the environmental impact of plastic polymers in the environment. Similarly, the use of chemical treatment may be avoided if the optimized use of microbial species yields positively during microplastic degradation. The use of microbial species is also considered environmentally safe.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.09.043.

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