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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.

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 clean-up 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

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