



Polyethylene Terephthalate (PET) degrading soil bacteria: an overview

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ABSTRACT

Despite their important role in food safety, medical safety, and other industries, plastic pollution remains one of the world's leading environmental issues. One particular plastic is Polyethylene terephthalate (PET), which has a relatively poor biodegradability. There are various traditional ways of managing plastic waste, but it takes years for plastic debris to degrade completely. Numerous conventional approaches have been proposed, but some pose environmental risks, prompting researchers to explore additional options. Using bacteria (e.g., *Ideonella sakaiensis* and *Thermobifida fusca*) to degrade plastics has been considered an alternative, sustainable approach to plastic waste management. In this paper, we briefly provide an overview of the role of plastics, their impact on the environment, their management, and the prospect for PET-degrading bacteria as one of the sustainable approaches in plastic, specifically PET, management.

KEYWORDS: Plastic degradation, *Ideonella sakaiensis*, *Thermobifida fusca*, Plastic waste management

INTRODUCTION

In the Philippines, plastic pollution remains one of the country's leading environmental issues. Around 2.7 million metric tons of plastic are generated yearly, 20% of which go to the world's oceans from local rivers and waterways neighboring areas where Filipinos reside (The World Bank, 2021). As a consequence of pollution, plastic's chemicals and toxic components are released into the soil, water, and air, which pose significant threats to the environment, humans, and marine life. Approximately 300 million metric tons of plastic are generated globally (Lai, 2022), and more than 82 million tons fall under Polyethylene terephthalate plastic (National Renewable Energy Laboratory, 2021).

Polyethylene terephthalate (PET) is a widely used type of plastic that is both light and durable, making it a significant component in manufacturing various commercial items such as bottles and food packaging. However, a concerning characteristic of this type of plastic is its poor biodegradability. In its backbone, PET contains single carbon bonds that are minimally reactive, making it difficult for bacteria to consume and degrade (Hiraga *et al.*, 2019). Other factors that make PET nearly resistant to biodegradation are the plastic's surface's hydrophobicity and its high molecular weight (Urbanek *et al.*, 2021). Natural biodegradation of these plastics is feasible. However, it can take long periods).

Aside from PET, other types of plastics are utilized worldwide, and people interact with the following polymers: High-Density Polyethylene (HDPE), Polyvinyl Chloride (PVC), Low-Density Polyethylene (LDPE), and polystyrene. The HDPE is a material that is resistant to moisture and is a primary component in detergent bottles, piping systems, and toys (Dusunceli & Colak, 2006; Hardin, 2021). Polyvinyl Chloride (PVC) is a polymer resistant to weathering and chemical degradation, making it suitable for construction and medical applications. While PVC is commonly found in plumbing pipes, oxygen masks, and IV fluid bags, it is known to secrete or leach dangerous chemicals such as lead and vinyl chloride (Hardin, 2021; Lieberzeit *et al.*, 2022). Low-density polyethylene (LDPE), a softer version of HDPE, is often used in the food industry as it is waterproof and convenient. These plastics are mostly applied in cling wraps, resealable bags, bubble wraps, and grocery bags (Jordan *et al.*, 2016; Hardin, 2021). Polystyrene, or styrofoam, is a low-cost insulator for food packaging and the construction industry. These polymers are usually found in cups, takeout containers, product packaging, and building insulation (Wünsch, 2000; Hardin, 2021). Similarly to PVCs, polystyrene is considered toxic due to its secretion of styrene, a known neurotoxin.

Using bacteria to degrade plastics has been considered an economical method as it utilizes an effective, sustainable strategy by using microbial processes and enzymes to perform their function (Taniguchi *et al.*, 2019). Utilizing bacteria to

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degrade plastics is also a viable method as these microorganisms thrive in conditions that do not require extensive conditioning, as bacteria can both inhabit an aerobic and anaerobic environment. Some studies have found that *Bacillus* and *Paenibacillus* sp. could degrade PETs (Park & Kim, 2019). Bacteria are also naturally occurring, which makes it more sustainable and attainable as a plastic degrading mechanism. Yoshida *et al.* (2016) discovered the bacterium *Ideonella sakaiensis* from Japan, which can degrade PET by synthesizing enzymes capable of hydrolyzing PET (Yoshida *et al.* 2016). The discovery of this type of bacteria is significant as plastic is a compound that can persist in the environment for hundreds of years. Discovering bacteria that can synthesize and degrade PET could potentially lead to developing more sustainable methods for plastic waste management and help mitigate the environmental impact of plastic pollution. Nevertheless, in-depth research is needed to fully understand the capabilities and limitations of plastic-degrading bacteria and their potential impact on plastic pollution.

In this paper, we provide an overview of the role of plastics, their impact on the environment, their management, and the prospect of PET-degrading bacteria as one of the approaches in plastic, specifically PET management.

ROLE AND TYPE OF PLASTICS

Despite plastics' adverse effects on the environment, their role in food safety, medical safety, and other important aspects of life is undeniable. Plastics possess a wide range of physical, chemical, and mechanical properties, making them appropriate materials for various applications. For the physical properties of plastics, it is a good material for absorbing moisture. This material is permeable to gasses and liquids, which are considered advantageous in suitable packaging, water resistant, and low in density, making them lightweight compared to metals (Xometry, 2023). For their chemical properties, plastics can be resistant to numerous chemicals, which makes them a good storage option for various chemicals in the industrial setting. However, plastics are sensitive to heat and light, which would result in degrading over prolonged exposure (Plastic Soup Foundation, 2020; Xometry, 2023). Furthermore, for its mechanical properties, plastics can deform without breaking, a property known as plasticity. Unlike metals, plastics have a low melting point, which makes them easily molded into different shapes and forms. The different types of plastics can range from soft and flexible to hard plastics, depending on the production process (Tomero, 2023; Osborne Industries, 2024). These properties' availability and low cost make them attractive and prospective materials for various applications ranging from industrial purposes to the healthcare industry. Plastics play a significant role in the food industry and are widely used in food production, packaging, and distribution. However, the extensive use of plastics in the food industry contributes to health concerns and the environmental issue of plastic pollution (FoodPrint, 2024; Mekitec, 2024).

Generally, plastics are integral to the construction industry as they offer durability, versatility, and cost-effectiveness. They are

used in various applications and materials, such as insulation, pipings, and wirings (Kramer, 2021). In the medical field, plastics provide essential barriers against infections, and medical devices such as gowns, catheters, and more ensure safety, efficiency, cost-effectiveness, and hygiene standards within the medical community (Dai A Industry, 2023). The automotive industry uses PET to produce lighter and more aerodynamic parts. Some textile manufacturers have also started to use PET in their fabrics.

Thus, instilling knowledge of different oil-based polymers is crucial to understanding their primary impact on the environment and the health factors associated with their usage and disposal. The reality of plastic-dependent earth would not change in the long run, as humanity has significantly benefited from plastics. While finding an alternative to these materials is possible, its disposal becomes one of plastics' greatest challenges.

Plastics are synthetic materials constructed from various organic polymers that can be molded or shaped into different forms and shapes (Thompson *et al.*, 2009). The main constituent of these polymers is petroleum, which contributes to its unique qualities such as versatility, durability, low cost, and resistance to corrosion and several chemicals (Ritchie & Roser, 2018). Because of their availability, plastics have become ubiquitous in modern society. Their applications and contributions range from packaging to construction, healthcare, and electronics. Over the years, plastics underwent significant changes and improved production methods, materials, and properties. Today, various types of plastics in the market vary widely depending on the type of polymer used and the manufacturing process, contributing to a unique range of characteristics and applications (Bahraini, 2022).

Polyethylene terephthalate (PET) is a polymer commonly found to produce consumer goods such as water bottles, soft drinks, and other beverages (Nisticò, 2020). In addition, this type of plastic is also used in food packaging, textiles, and electronics. This type of plastic is a thermoplastic polymer derived from petroleum. According to Ji (2013), PETs are known for their transparency, strength, and lightweight dress. Therefore, it is an excellent choice for packaging. PET polymers are formed through ethylene glycol and terephthalic acid polymerization, also known as dimethyl terephthalate. The resulting polymer can be molded into different forms by adding heat (Daubeney *et al.*, 1954; Koshti *et al.*, 2018). Even though this plastic is described as recyclable due to its versatility and widespread use, it has led to several concerns due to its environmental impact (Jankauskaite & Lygaitis, 2008). Specifically, PET plastics significantly contribute to plastic waste in landfills and oceans.

Polyethylene (PE) is a polymer also produced from petroleum (Ronca, 2017). Being a thermoplastic polymer, it has a unique application in consumer goods and the industrial area (Peacock, 2000). According to Paxton *et al.* (2019), the applications of polyethylene expand to a wide range of products such as plastic bags, food packaging, piping systems, and medical devices. Due to its low production cost, it is a desired choice in several industries. In contrast to PETs, polyethylene is

produced through a polymerization process that involves ethylene. Ethylene molecules are chemically bonded to form long chains of polymer molecules (Zhong *et al.*, 2018). This polymerization process may produce two subtypes of polyethylene: High-Density Polyethylene (HDPE) and Low-Density Polyethylene (LDPE) (Patel, 2016). While both are polyethylene polymers, their properties and uses differ due to their molecular properties. High-density polyethylene (HDPE) has a higher density and a more complex crystalline structure than its counterpart. This structure gives HDPEs greater rigidity, strength, and resistance to impacts and environmental stress (Kanagaraj *et al.*, 2007). Because of its strength, this type of polyethylene is used to produce pipes, bottles, containers for liquids and chemicals, and automotive parts (Shell Polymers, 2020). On the other hand, Low-Density Polyethylene (LDPE) has a lower density than HDPE due to its branched molecular structure. Unlike HDPE, LDPE has greater flexibility, ductility, and heat-sealing properties (Jordan *et al.*, 2016). With this, LDPE is commonly used in films, bags, food packaging, and other objects that require flexibility (tubing, insulations, etc.). With the different properties of LDPEs and HDPEs, they both have different recycling processes. For example, HDPEs take longer to disintegrate. However, they are more recyclable than LDPEs, where they deteriorate faster but are more difficult to recycle (Yashoda, 2016).

Polystyrene, produced from styrene monomers, is a thermoplastic material with several desirable properties, such as its lightweight dress, stiffness, and insulation capability (Wünsch, 2000). Unlike the polymers mentioned above, polystyrene can insulate contents that are packaged within it. As a result, it has several applications in the food, construction, and transport industries. It is usually utilized in cups, trays, adhesives, sealants, and coatings (Tefamariam, 2022). Produced through suspension polymerization, styrene monomers (a liquid hydrocarbon derived from petroleum) are dispersed in water. The resulting polymer particles are then separated from the suspension and dried to produce polystyrene beads or pellets. These pellets can be further processed into various forms, such as foam boards, cups, trays, and packaging materials, through extrusion, injection molding, and thermoforming (Kaufman, 1968; Brooks, 2010). However, according to a paper by Maharana *et al.* (2007), polystyrene has come under scrutiny for its environmental impact due to its contributor to plastic waste and pollution as it is both non-recyclable and non-biodegradable, just like all petroleum-based plastics.

LIFE CYCLE ASSESSMENT OF PLASTICS

Life Cycle Assessment is important in assessing plastics' environmental impacts and identifying future improvement opportunities. According to Finnveden *et al.* (2009), Life Cycle Assessment (LCA) is a methodology used to evaluate the environmental impacts of products and processes across their entire life cycle. The life cycle described here is from raw materials and production extraction to the product's "end of life" or recycling (Hellweg & Milà i Canals, 2014). Therefore, this methodology can assess the environmental

impacts of specific materials, such as plastics, by considering their production, usability, duration of use, and until their disposal. The LCA of plastics involves considering the range of environmental impacts, which usually encompasses the energy used to produce these, water consumption, land use, toxic chemicals released during production, and greenhouse gas emissions (Walker & Rothman, 2020). By understanding LCA studies regarding plastics, improvements can be made to reduce the carbon footprint and environmental impact and utilize more sustainable raw materials. LCA studies, such as those conducted by Santos *et al.* (2021), have been conducted on a range of plastic products and materials, including polyethylene, polystyrene, PVC, and PET. These studies have identified opportunities to reduce the environmental impacts of plastics by improving several aspects, from the production process's efficiency to the end-of-use management.

PLASTIC POLLUTION

Plastic's low cost makes it one of the world's most widely utilized and readily available materials today, leading to an enormous surge in plastic manufacture and generating an issue on a global scale. Attributed to plastic's non-biodegradable nature, it poses long-term social, economic, and ecotoxicological risks for both surface and marine life and the environment. Thus, plastic waste pollutes the environment and threatens ecosystems (Taniguchi *et al.*, 2019).

The media has recently focused much attention on the millions of tons of plastics littering the world's oceans. However, the plants, animals, and humans who live on land may be more at risk from plastic pollution. In waste-to-energy facilities, just a tiny portion of the plastic dumped daily is recycled or burned. Instead, much of it is disposed of in landfills. It might take up to 1,000 years to degrade and release potentially harmful materials into the soil. According to Lin *et al.* (2020), terrestrial microplastic pollution has caused a decline in species that dwell below the surface, such as mites, larvae, and other microscopic organisms that maintain the fertility of the land. Plastics also have the potential to release toxic chemicals into the soil, where these chemicals can then seep into nearby water sources, including groundwater and the ecosystem (UN Environment Programme, 2018).

Plastics have caused much damage to marine ecosystems. Several studies have reported that ingestion and entanglement are the top ways that plastics cause harm to marine life (Derraik, 2002). For example, in the Mediterranean, 49,454 organisms were reported to have ingested plastic, and 44 species were entangled in plastics (Anastasopoulou & Fortibuoni, 2019). In addition, both macroplastics and microplastics have also been reported to be dangerous to marine life (Li *et al.*, 2016).

Besides its effects on land and marine life, plastic has also been found to affect human health. Plastic additives, such as phthalates, bisphenol A, and polybrominated diphenyl ethers (PDBE), are considered carcinogens. However, it is generally agreed upon that they alter the endocrine system. It was also reported that

there is less data on human reproduction or development effects than on animals (Kumar, 2018). A study from Bangladesh reports that the previously mentioned plastic additives, as well as antinimitoxide, have adverse effects on human health. Plastics are also linked to issues in the eyes, respiratory system, and liver, as well as cancers (Proshad *et al.*, 2017).

PLASTIC POLLUTION IN THE PHILIPPINES

The Philippines is considered one of the world's worst offenders of plastic pollution (Garcia *et al.*, 2019). The country is the third largest contributor to plastics in terms of marine plastic pollution, with an estimated 0.28-0.75 million tonnes of plastic waste entering the oceans (Sea Circular Project, 2020). According to Pathak *et al.* (2023), one practice seen as an alternative to waste disposal is burning waste. This practice is seen as prevalent among the marginalized sector due to the lack of proper waste disposal and management. Although highly discouraged by the local government, this solution is considered the most efficient way as communities do not have access to waste disposal resources, insufficient management facilities, and lack of enforcement coupled with the public's lack of awareness.

One main factor that collectively contributes to the significant plastic waste issue of the Philippines is the sachet economy, which runs rampant among the general population. According to Enerva (2022), a sachet is a single-use plastic pouch that is used to store condiments in first-world countries; however, in developing countries, these plastic pouches are used to store household necessities from sauces and seasonings to detergents, shampoos, and soaps. Producing sachets is inexpensive for manufacturers, and for low-income households, this allows them to purchase small quantities of products at an affordable rate. The sachet economy is described as the continuous and widespread use of these small, single-use plastic packets that are readily bought in convenience stores and in local stores known as *sari-sari* stores, which are prevalent in the country due to factors like limited income, high demand, large families, and rising consumerism (Sarmiento, 2018).

According to the University of Portsmouth (2023), an estimated 3 million tonnes of plastic are produced yearly, with single-use plastic sachets accounting for 52% of this production in the Philippines. The sachet economy poses significant environmental challenges, particularly in soil health, where soil contamination is risky. Specifically in the rural landscape, food production and cultivation are most affected due to plastic pollution, climate change, and biodiversity loss (Ibrahim *et al.*, 2020). Plastic pollution in rural settings is less studied than in urban environments. Since the Philippines is an agriculturally rich country, it is fundamental to determine the effects of having a plastic-reliant economy in a rural setting. The rural environment of the Philippines is degrading due to the mechanisms of the current economy, such as reliance on fossil fuels, depletion of natural resources, effects of climate change, and waste pollution. All these factors contribute to the marginalization of the rural setting (Pain & Hansen, 2019).

PLASTIC WASTE MANAGEMENT

There are various traditional ways of managing plastic waste, but it takes years for plastic debris to degrade completely. Numerous conventional approaches have been proposed, but some pose environmental risks, prompting researchers to explore additional options, including microorganism-based methods for appropriate, cost-effective, and eco-friendly ways to manage plastic waste.

One common method of disposing of PETs is through burning, which may be fast but environmentally taxing and is not allowed in the country. Aside from being banned in the country (Ecological Solid Waste Act, 2000), burning trash like plastics (food packaging and containers) can release toxic chemicals such as nitrogen oxides, sulfur dioxide, and volatile organic compounds. One of the major contributors of greenhouse gases is open waste burning, as vast amounts of carbon dioxide and carbon monoxide are released into the atmosphere.

Another method is through reusing PET bottles in the form of *ecobricks*. An *ecobrick* is a PET bottle packed with single-used plastics (tetra packs, food packaging, etc.) up to a specific density, which would serve as a backbone for construction materials, furniture, and many more structures (Antico *et al.*, 2017). This form of upcycling takes advantage of the property of plastic as a durable, waterproof, and insulating material (Cleanaway, 2018). While *ecobricks* offer a ready-made solution to the issue of plastic waste, it would not stand the test of time. After 2-3 years, silicone joints that join *ecobricks* weaken and fail. In addition, concerns have been raised that in a hot and humid environment, inorganic materials from plastics may leech into the surrounding environment (Singh, 2020).

Conventional methods have been developed to treat nonbiodegradable plastic waste (NPW). These methods include mechanical and chemical recycling, landfill complemented by incineration, and pyrolysis. However, each technique has a restricted application range and consequently hits a particular bottleneck. The incineration of plastics uses much energy and emits dangerous byproducts (Ashworth *et al.*, 2014). Yang *et al.* (2012) stated that by-products like CO₂, acidic gasses (sulfur oxides), persistent organic compounds (dioxins and furans), heavy metals, and particulate matter are hazardous and can contribute to global warming as well as several health issues, such as respiratory symptoms, lowered lung function, and a higher risk of developing cancer. Another conventional method commonly practiced for managing plastic waste is landfilling. Unfortunately, nonbiodegradable plastics take up more and more land due to the increasing amount of plastic being disposed of and only disintegrate slowly in landfill settings. It also imposed many health concerns. As a result, landfilling has been deemed the least ideal management technique and is subject to numerous restrictions (Okan *et al.*, 2019).

MICROORGANISMS IN PLASTIC WASTE MANAGEMENT

A possible alternative method for the degradation of plastics is using microorganisms such as fungi and bacteria, which have sustainable and eco-friendly properties (Zeenat *et al.*, 2021). Fungal species, namely *Fusarium solani*, *Spicaria* spp. *Alternaria solani*, and *Aspergillus flavus*, were reported to have plastic-degrading properties (Ibrahim *et al.*, 2011). *Bacillus* sp. and *Paenibacillus* sp. were reported to degrade plastics, specifically polyethylene microplastics (Park & Kim, 2019). *Ideonella sakaiensis* has also degraded PET (Yoshida *et al.*, 2016). Nonetheless, while the most well-known plastic-degrading bacteria are from the genera *Pseudomonas*, *Bacillus*, and *Idionella*, little research has been conducted in the Philippines and worldwide.

Biodegradation is the process behind the microbial degradation of plastics. Biodegradation is the natural process in which a specific species of bacteria breaks down an organic substance into more minor constituents or simpler compounds, namely, water, carbon dioxide, and biomass (Roohi *et al.*, 2017). In plastic polymers composed of organic substances (petroleum), certain species of bacteria are known to break down the chemical compounds that bond polymer chains. Doing so converts the broken-down chain into smaller molecules that can be metabolized for energy and growth (Ghosh *et al.*, 2019; Elahi *et al.*, 2021). In addition, the enzymes produced by bacteria (lipases, proteases, etc.) are known to break the chemical bonds in plastics.

The degradation of plastics through microbial and enzymatic means is a promising approach, as the depolymerization of waste can produce products such as carbon dioxide, water, and biomass. The biodegradation of plastics through enzymatic methods involves the secretion of extracellular enzymes that attach to the surface of the plastic, which would be hydrolyzed into short polymer intermediates. The bacteria consider these intermediates a rich carbon source, hence its byproduct of carbon dioxide (CO₂). Numerous microorganisms, including fungi and bacteria, have been identified to metabolize petroleum-based plastics, mostly in an *in vitro* environment (Jumaah, 2017). However, it is important to note that not all types of plastics are easily degradable by enzyme-producing bacteria. It would take plastics thousands of years to degrade or break into smaller fragments. It is also important to consider the specific conditions where the specific bacteria would thrive and reproduce. Certain requirements must be met for an optimal biodegradation setting (temperature, moisture, humidity, etc.) (Yuan *et al.*, 2020).

PET-DEGRADING BACTERIA

Various strategies have been developed to alleviate plastic pollution caused by PET. A recent study by Edwards *et al.* (2022) has shown that the synergy of a consortium of five bacterial species can potentially degrade PET successfully. A novel discovery of EstB and PETase hydrolyzing the Polymer PET

was also observed, proving its efficacy. It was also identified that *Pseudomonas* spp. has an encoded EstB PETase, which can be a source of robust degrading capacity.

Most existing studies regarding PET-degrading bacteria utilized numerous strains or employed the synergy of bacterial species, allowing researchers to compare and observe relative characteristics of degradation capabilities. This approach is effectively exhibited in the study of Lee *et al.* (2021), wherein the degradation rate of co-culture of two bacterial species was compared to the degradation rate of monocultures to compare and maximize possible biodegradation performance. For instance, the monoculture of *Thermobifida fusca* yielded a 6.697% degradation rate, which is relatively higher compared to the co-culture of *Thermobifida fusca* and *Ideonella sakaiensis* of 3.229%, and this also yielded higher degradation than the monoculture of *T. fusca* alone which only yielded 0.4325% degradation rate.

Therefore, findings from studies like the study above provide us a baseline to compare the performance of certain species based on specific properties they distinctly possess that may have been observed from such mechanisms such as carbon dioxide utilization, biofilm formation, enzyme activity assays (Edwards *et al.*, 2022; Fernández *et al.*, 2022). However, most of the findings are still inconclusive. They may require further studies to prove their specific capacity to degrade PET and other plastics.

Generally, PET-degrading bacteria possess enzymes such as PETase that play a role in the degradation of polyethylene terephthalate (Urbanek *et al.*, 2021). The molecular mechanism of degradation relies on the ability of PETase to bind to the plastic surface, such as in the case of *Ideonella sakaiensis*, which would lead to a two-step degradation process (Burgin *et al.*, 2024). The first step involves the ability of the enzyme PETase to bind to the PET surface that would, cleave the ester bond, and lead to the generation of PET chains that have two different terminals: terephthalic acid (TPA) and ethylene glycol (EG). Following this step, the PET chains are further degraded into monohydroxyethyl terephthalate (MHET) monomers, a key intermediate in the enzymatic degradation of polyethylene terephthalate (Jerves *et al.*, 2021).

To intensively grasp the degradation capacity and comprehend the mechanism of the already known bacterial species that exhibit potential contribution to developing synergistically effective bacterial strains, examining these species individually and discussing important findings relevant to each before arriving at a collective understanding of PET-degrading bacteria. Through this, we may also understand what makes a particular bacterial species capable of degrading PET or plastics effectively.

Ideonella Sakaiensis

In 2016, researchers in Japan isolated a bacterium from soil samples in a PET recycling factory. They discovered its potential for biodegradation (Yoshida *et al.*, 2016). The gram-stain-

negative, aerobic, and non-spore-forming bacterium was later identified as *Ideonella sakaiensis* (Tanasupawat *et al.*, 2016), which was a novel species of *Ideonella* based on a polyphasic taxonomic study. This bacterium can metabolize PET waste and convert it into a carbon and energy source. The bacterium *I. sakaiensis* produces the key enzymes PETase and MHETase. PETase converts PET into MHET (mono-(2-hydroxyethyl) terephthalate), the reaction intermediate. Meanwhile, MHETase can hydrolyze MHET into the PET monomers, terephthalic acid, and ethylene glycol. However, the slow degradation rate is measured at approximately six weeks for a thin, low-crystallinity PET film (Henderson, 2020).

Walter *et al.* (2022) have shown that *I. sakaiensis* can biodegrade commercial PET materials. According to Burgin *et al.* (2024), the ability of the bacterium to extracellularly depolymerize PET involves a two-enzyme system: PETase and MHETase. PETase is the enzyme that directly breaks down PET into bis-hydroxyethyl terephthalate (BHET) and mono-hydroxyethyl terephthalate (MHET). MHETase then cleaves MHET into ethylene glycol and terephthalic acids, which would be used as a substrate for *I. sakaiensis* metabolism (Hachisuka *et al.*, 2021).

Thermobifida Fusca

Thermobifida fusca, as mentioned, is already a known bacterium with an emerging interest in its potential use in PET degradation, with its proven efficiency through certain properties. This bacterium is described as a thermophilic, filamentous, and spore-forming bacterium known for its role in the degradation of plant cell walls (del Pulgar & Saadeddin, 2013). *T. fusca* is a gram-positive, rod-shaped bacterium classified as an obligate aerobe that optimally grows at 50-55°C. The cellulolytic system of this bacterium is well studied as it produces a variety of extracellular enzymes, including cellulitis, mannanase, and lytic polysaccharide monooxygenases (del Pulgar & Saadeddin, 2013).

Thermobifida fusca was studied for its biodegrading properties, specifically its cutinase enzyme (Furukawa *et al.*, 2019). The thermostable cutinase from *T. fusca* (TfCut2) can degrade PET into its terephthalic acid monomer. The enzyme can hydrolyze low-crystallinity PET without high reaction conditions. However, for high-crystallinity PET, the hydrolysis observed was very minimal. The researchers also discovered that with the addition of a cationic surfactant, the degradation rate of PET increased due to the electrostatic interactions that attracted more enzymes.

The physiological and cellulolytic features of *T. fusca* are noteworthy mainly because of its capacity to withstand high temperatures by producing thermostable cellulases due to its endospore, which enables it to tolerate extreme conditions aside from having high thermostability, it is also highly efficient in a wide pH range (Hegde & Dasu, 2014). Based on the study conducted by del Pulgar & Saadeddin (2013), the monoculture of *T. fusca* has a reported degradation rate of 6.697%, showing the highest degradation rate among other monocultures of

bacterial species being compared. Additionally, a recent study conducted by Ko *et al.* (2024) yielded favorable results on the degradation rate of *Thermobifida fusca* FXJ-1, which is at 52.53% to commercially available biodegradable plastics also collectively referred to as PBAT-PLA-TPS. This further confirms the capacity and potential of *T. fusca* to degrade plastics of various kinds.

Other PET-degrading Soil Bacteria

Some bacteria were also isolated from petroleum-polluted soil and tested for lipase activity (Roberts *et al.*, 2020). The study used a bacterial consortium of *Pseudomonas sp.* and *Bacillus sp.* for PET biodegradation. As a result, the synergy of the five strains of bacteria within the two species was discovered to be effective in metabolizing and fully converting bis(2-hydroxyethyl) terephthalic acid (BHET) into the monomers terephthalic acid (TPA) and ethylene glycol. In addition, the presence of the by-products of PET hydrolysis was also observed through 1H-nuclear magnetic resonance (1H NMR) analysis. *Bacillus sp.* and *Paenibacillus sp.* were also reported to degrade plastics, specifically polyethylene microplastics (Park & Kim, 2019).

Fungi have been also found to have potential biodegrading capacity due to possessing unique properties such as hydrophobins, which act as a coating for hyphae, allowing them to penetrate three-dimensional substrates, and enzymes for the detoxification of pollutants (Sánchez, 2020). Fungal species, namely *Fusarium solani*, *Spicaria spp.*, *Alternaria solani*, and *Aspergillus flavus* were reported to have plastic-degrading properties (Ibrahim *et al.*, 2011; Saleem & Hasan, 2017).

In their study published in 2019, Malafatti-Picca *et al.* (2023) reported 4 potential fungal strains that could degrade PET by converting PET nanoparticles into terephthalic acid [C₆H₄(CO₂H)₂]. These strains were identified to be *Curvularia trifolii* CBMAI 2111, *Trichoderma sp.* CBMAI 2071, *Trichoderma atroviride* CBMAI 2073, and *Cladosporium cladosporioides* CBMAI 2075. To confirm their findings, a fermentation assay found that at 12 ppm or higher concentrations, the fungi released terephthalic acid when combined with PET. Weight loss measured with a weight loss assay, material roughness seen with a scanning electron microscope, and band modification and the presence of enzymatic activities from lipase, esterase, and cutinase measured with Fourier-transformer infrared (FTIR) spectroscopy further confirmed the biodegradation of the PET after exposure to the fungi. In addition, they found that PET with higher crystallinity was more resistant to the effect of the enzymes.

A review of known PET-degrading fungi was done by Ahmaditabatabaei *et al.* (2021). They found that some strains of fungi degrade PET into low molecular weight oligomers or monomers like BHET (bis(2-hydroxyethyl)terephthalate) and MHET (mono(2-hydroxyethyl)terephthalate). These are bonded by ester bonds, which some species of fungi can cleave using lipases, esterases, and cutinases. These enzymes modify the characteristics of PET, such as its surface changed by esterases, and wettability changed by lipases. Cutinases, on

the other hand, catalyze PET degradation. Cutinases that can degrade PET with low crystallinity were found in *Aspergillus oryzae*, *Aspergillus nidulans*, *Penicillium citrinum*, *Humicola insolens*, *Fusarium solani*, *Fusarium solani* pisi, and *Fusarium oxysporum*. The cutinases from these fungal species were found to break down the PET into their monomers (BHET and MHET), then to ethylene glycol $[(CH_2OH)_2]$ and terephthalic acid.

ENZYMES INVOLVED IN PET DEGRADATION

Scientists have discovered 27 enzymes that can degrade synthetic polymers (Danso *et al.*, 2019). Among these, the enzymes primarily associated with PET breakdown are classified as serine hydrolase. These include the enzymes cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3), and carboxylesterases (EC 3.1.1.1) (Roth *et al.*, 2014). The catalytic trio of these enzymes comprises aspartate, histidine, and serine residues, and they display the distinctive α/β -hydrolase shape. Furthermore, it is possible that they have many disulfide connections that are aided by cysteine residues, which improve heat stability and allow for targeted binding to PET.

Yoshida *et al.* (2016) conducted a study wherein they isolated *Ideonella sakaiensis* from soil samples in a PET recycling factory and discovered its potential for biodegradation. This bacteria can metabolize PET waste and convert it into a carbon and energy source. The bacterium *I. sakaiensis* produces the key enzymes PETase and MHETase. PETase converts PET into MHET (mono-(2-hydroxyethyl) terephthalate), the reaction intermediate. Meanwhile, MHETase can hydrolyze MHET into the PET monomers, terephthalic acid, and ethylene glycol. However, the slow degradation rate is measured at approximately six weeks for a thin, low-crystallinity PET film (Henderson, 2020).

A study by Danso *et al.* (2018) found new thermostable enzymes that may degrade PET. They developed a hidden Markov model to find potential PET-degrading genes and enzymes using already known sequences, allowing them to study the taxonomic relationships of the PET-degrading genes and enzymes. Their study started with 16 Gb of sequence information from marine and terrestrial sources, from which they found 504 potential novel enzymes and 349 candidate genes and enzymes. By grouping the enzymes based on the similarities of their amino acids, the study found that PET hydrolases were found in *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*, with *Bacteroidetes* being the major host for PET degrading enzymes in the marine environment, and *Actinobacteria* in the terrestrial environment. From the enzymes they found, they further studied PET₂, 5, 6, and 12, and through further tests, found that PET₂ and 6 were thermostable. PET₂ was stable at temperatures up to 90°C, and its residual activity was still greater than 50%.

In 2019, *Thermobifida fusca* was studied for its biodegrading properties, specifically its cutinase enzyme (Furukawa *et al.*, 2019). The thermostable cutinase from *T. fusca* (TfCut2) can degrade PET into its terephthalic acid monomer. The enzyme

can hydrolyze low-crystallinity PET without high reaction conditions. However, for high-crystallinity PET, the hydrolysis observed was very minimal. The researchers also discovered that with the addition of a cationic surfactant, the degradation rate of PET increased due to the electrostatic interactions that attracted more enzymes.

Some bacteria were also isolated from petroleum-polluted soil and tested for lipase activity (Roberts *et al.*, 2020). The study used a bacterial consortium of *Pseudomonas sp.* and *Bacillus sp.* for PET biodegradation. As a result, the synergy of the five strains of bacteria within the two species was discovered to be effective in metabolizing and fully converting bis(2-hydroxyethyl) terephthalic acid (BHET) into the monomers terephthalic acid (TPA) and ethylene glycol. In addition, the presence of the by-products of PET hydrolysis was also observed through 1H-nuclear magnetic resonance (1H NMR) analysis.

POTENTIAL SOURCE OF PET-DEGRADING BACTERIA

Landfills are a commonly used method for disposing of waste materials, including plastics. As plastic waste continues to amass in these sites, various research has been aimed at investigating the potential for plastic-degrading bacteria to cultivate in landfills. Landfills are a complex ecosystem involving environmental factors, such as moisture, temperature, and nutrient availability. Over time, bacteria and other microorganisms can inhabit and adapt to harsh conditions and develop new metabolic pathways to break down organic materials (Munir *et al.*, 2018). Plastic waste in landfills can provide a new source of carbon and energy for these microorganisms, potentially selecting bacteria that can break down plastics. For instance, Yoshida *et al.* (2016) isolated and identified a bacterial strain that could degrade polyethylene terephthalate (PET). The researchers found that the bacteria produced enzymes that could break down PET into its constituent monomers, which could then be further metabolized by other microorganisms in the landfill. Landfills can serve as a source for plastic-degrading bacteria. As plastic waste accumulates in these sites, microorganisms adapt to the conditions and develop new metabolic pathways to break down these materials.

Several studies have investigated the growth conditions of bacteria that can degrade PET. The study of Qi *et al.* (2021) determined that *Bacillus sp.*, *Aspergillus sp.*, and *Spirulina sp.* have demonstrated PET biodegradation potential. *Bacillus sp.* achieved a 43.05% weight reduction of pretreated PET after 30 days, and *Spirulina sp.* degraded 48.61% of PET microplastics. Additionally, in the systematic review conducted by Fernández *et al.* (2022), it was stated that three bacterial strains that were isolated from plastic-contaminated sites, namely *Priestia aryabhattai*, *Bacillus pseudomycoides*, and *Bacillus pumilus* were able to degrade PET powder by over 65% in 18 days and PET sheets by over 65% in 28 days. It was determined that these strains could grow on PET alone without any additional carbon or energy source. It is determined that the optimal growth conditions for PET-degrading enzymes are

usually 30-40 °C, where PET-degrading bacteria can rely on PET as the sole carbon source at temperatures around 30-40 °C (Zhao *et al.*, 2023). Pretreatment of PET, nutrient availability, and microbial consortia can enhance the degradation rate. PET-degrading bacteria have been isolated from environments like soil, plastic-contaminated sites, and marine sediments.

CULTURE MEDIA FOR PET-DEGRADING BACTERIA

The increasing accumulation of polyethylene terephthalate (PET) in the environment has become a significant ecological concern due to its widespread use and resistance to natural degradation. PET-degrading bacteria can break down PET into less harmful byproducts, thus offering a potential solution to mitigate plastic pollution. Using effective culture media for PET-degrading bacteria is important for optimizing their growth and degradation capabilities. The culture media must provide the essential nutrients and conditions that promote bacterial proliferation and enhance their enzymatic activity on PET substrates. This study utilizes two trials that involve the use of two mediums, namely: Nutrient broth and Bushnell Haas Agar.

Nutrient Broth is a widely utilized medium used in culturing undemanding microorganisms (LabMal Academy, 2022). It is a general-purpose medium suitable for cultivating a wide variety of non-fastidious microorganisms with basic nutritional requirements (Aryal, 2022). This medium appears to be liquid at room temperature, unlike nutrient agar, a solid medium containing agar that solidifies at room temperature. While nutrient agar is mainly used for the isolation and cultivation of organisms that allow for the formation of distinct colonies, the nutrient broth is more suitable for the growth of fastidious organisms (LabMal Academy, 2022; Sandle, 2019). The ingredients of this culture media include peptones, beef extract, yeast extract, and Sodium Chloride (NaCl). These components provide essential nutrients for microbial growth (Tankeshwar, 2024). The presence of turbidity usually indicates bacterial growth in this type of media due to microbial multiplication. One of the methods for this study is adapted from the methodology of Kathiresan (2003) entitled “*Polythene and Plastics-degrading microbes from the mangrove soil*”. The study used pre-weighed plastic discs (1 cm diameter) made from polythene bags and disposable plastic cups to assess microbial degradation. These discs were aseptically transferred into conical flasks containing 50 mL of culture broth medium. Different bacterial species were inoculated into separate flasks, using nutrient broth for bacteria. Control flasks with plastic discs in a microbe-free medium were also prepared. Each treatment had four flasks placed on a shaker for one month. After shaking, the discs were collected, washed with distilled water, shade-dried, and reweighed. The weight loss of the plastics was then calculated from the initial and final weights.

Bushnell Haas Agar is a medium used to examine hydrocarbon deterioration by microorganisms. This media type examines fuels for microbial contamination as they contain nutrients necessary for growth, excluding hydrocarbons. For the separate

trial, the methodology involving Bushnell Haas Agar was adapted from the study of Nakei *et al.* (2002), which involves assessing the degradation of different types of plastics by bacterial isolates using Bushnell Haas mineral agar medium. Ground polyethylene (PE) powder sieved through a 0.6 mm sieve was added to the mineral salt medium and mixed for 1 hour at 120 rpm. The medium was autoclaved and cooled before being dispensed into Petri dishes to solidify. Microorganisms were then inoculated onto these plates and incubated at 27 °C for up to 21 days. Periodic observations were made for clear zones around colonies, indicating plastic degradation. The diameters of the colonies and clear zones were measured to indicate the secretion of biosurfactants or other bacteria compounds that inhibit the growth of other microbes. A clear zone appears around the sample on the opaque agar the presence and size of this zone of inhibition indicates the antimicrobial activity of the sample and suggests the presence of hydrocarbon-degrading microbes (Vignesh *et al.*, 2016; Hisham *et al.*, 2019).

METHODS FOR CONVERTING PET INTO POWDER

Ball milling is widely used among different techniques to reduce the particle size of metals, ceramics, and polymers. However, reducing the size of polymers through milling presents a challenge due to the issues with repeated fracturing and cold welding of the polymer particles. To address this, it has been demonstrated that cryomilling, which involves milling at relatively low temperatures such as the temperature of liquid nitrogen (−196 °C), can effectively reduce the particle size of polymers (Zhu *et al.*, 2006; Giri *et al.*, 2014).

In the research study of Giri *et al.* (2014), PET powders were produced using cryomilling. This technology could avoid problems common to traditional processing techniques, like excessive viscosity and insolubility. The researchers used liquid nitrogen (LN₂) to mill the initial powders for cryomilling, creating a slurry by the milling balls. This kind of milling involves the powder interacting intimately with LN₂ and is called “cryogenic attrition.”

Another method for producing PET powders was illustrated in a study by Hussein *et al.* (2018). Their research combined 100 grams of PET fragments with 116 milliliters of ethylene glycol (EG) in a 4:1 EG to PET molar ratio, supplemented with 0.05% Nano-Magnesium Oxide acting as a catalyst. This mixture was depolymerized at the boiling point of EG for 40 minutes until complete depolymerization occurred. Heat treatment involved full condensation through refluxing in a bubble column reactor with zero material loss, utilizing a glass condenser cooled with water. Unreacted ethylene glycol was subsequently separated from the mixture, resulting in the final PET form.

BACTERIAL IDENTIFICATION TECHNIQUES

There are multiple ways to identify bacteria, from its gross morphology to its microscopic and molecular characteristics (Sloan *et al.*, 2017). Bacterial identification begins with examining the morphology of the bacterial colonies on the

surface of the agar. Colonies differ in texture, color, size, shape, and, in some cases, odor. This is beneficial for quick bacterial identification, but this technique falls short as some species have similar colony appearances. Its other characteristics must be inspected to more conclusively identify the colonies on an agar plate (Sousa *et al.*, 2013).

Gram staining differentiates Gram-positive (+) and Gram (-) bacteria. After staining, Gram-positive (+) bacteria will appear purple, and Gram-negative (-) bacteria will appear pink. To do Gram staining, a bacteria sample is placed on a slide and then heat-fixed. After this, the slide is stained with crystal violet, which stains all the bacterial cells with violet. A mordant, in this case, iodine, fixes the crystal violet onto the cells. 95% ethyl alcohol is then applied onto the slide, which washes the initial stain away from Gram-negative (-) cells, as they have thinner peptidoglycan walls than Gram-positive (+) bacteria. After the alcohol washing step, the secondary stain, safranin, is added to a slide, which is then taken up by the Gram-negative (-) bacteria, as the crystal violet from the cells has been removed. Gram staining is useful in identifying bacteria more specifically than gross colony morphology analysis, but other molecular techniques will be more specific and conclusive (Tripathi *et al.*, 2023).

Mass spectrometry (MS) is an analytical technique used in bacterial identification. These techniques enable rapid examination of biomarker ions, providing reliable information on bacterial characterization, even down to the sub-species level (Krásný *et al.*, 2013). The method most commonly employed is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), where ribosomal proteins and peptides in a purified culture are quantified by generating mass spectra. Since these proteins are notably unique to each bacterial species, the resulting mass spectra serve as distinct identifiers, facilitating precise identification of purified strains at both the genus and species levels. The analysis starts with isolating a set of strains grown under different cultivation conditions, then goes through a purification procedure to increase biomass to a suitable degree. Microbial cells are grown and then added to an appropriate organic matrix solution on a metal target plate, along with their crude extracts. The unlabeled spectrum of a novel strain is then identified using software tools, which typically consist of one or more machine learning algorithms (Mortier *et al.*, 2021).

Polymerase Chain Reaction, or PCR, is a laboratory technique used to produce copies or amplify a specific region of DNA. It is a process that involves three main procedures, namely, denaturation, annealing, and extension (Joshi & Deshpande, 2010). During the denaturation phase, the DNA strands are separated through heat. During annealing, primers specific to the target DNA sequence anneal or bind the primer DNA to the template DNA. In extension, the DNA polymerase synthesizes new DNA strands utilizing the primers as templates, which amplifies and reproduces the target DNA sequence (Jäger & Weiher, 2020). In species identification, the process of PCR can be used to amplify regions of interest in the bacterial DNA, such as the 16S ribosomal RNA (rRNA), which allows for the identification of bacterial species (Fukuda *et al.*, 2016).

According to Kim and Chun (2014), the 16S rRNA has been extensively used in the classification, and the identification of bacteria as ribosomal RNA genes has been considered standard phylogenetic markers in taxonomic studies. This specific gene is considered highly conserved among bacteria as its presence in its genomes and the slow evolutionary rate have made it an ideal sequence for phylogenetic analysis and taxonomic classification. The comparison of a complete 16S rRNA sequence with a database of known sequences allows for a specific and accurate approach to identifying a bacterial species (Watts *et al.*, 2017). The amplification of the 16S rRNA through PCR involves designing specific primers complementary to the target sequence that will be amplified. After the PCR process to amplify the specific gene and identify bacterial species based on sequence analysis, sequence data can be utilized to construct phylogenetic trees and further elucidate evolutionary relationships between bacterial taxa.

Phylogenetic Analysis is the study of the evolutionary development of a certain species. It is used to reconstruct the phylogeny of a certain group of organisms based on the similarities and differences in their genetic makeup or morphological characteristics (Patwardhan *et al.*, 2014). Phylogenetic analysis is presented in a phylogenetic tree, also known as a cladogram, which depicts the branching patterns of evolutionary relationships among the organisms under study (Helmenstine, 2020). Constructing a phylogenetic tree may utilize different comparisons, such as nucleotide sequences, the presence or absence of specific traits, or physical characteristics. With this, various algorithms can be inferred in a phylogenetic tree, such as a neighbor-joining tree that uses distance-based methods, a maximum likelihood tree, or a maximum parsimony tree (Challa & Neelapu, 2019).

RESEARCH PROSPECT AND OUTLOOK

In this fast-changing world and dynamic economy, many actions are needed to search for an economical and environmentally friendly solution to the plastic waste our world produces. As plastic waste has become a global environmental issue that threatens the health of our ecosystems and human well-being, it has become a challenge for us. With rapidly evolving technologies and improving sciences, plastic-degrading bacteria may become a promising solution to the issue of plastic waste accumulation in the environment. The overall landscape, including the relationship between plastics and microorganisms, is key to understanding the present case.

According to research conducted by Gao and Sun (2021), microorganisms have the ability to degrade plastics not only in the terrestrial environment but also in the marine ecosystem. As plastic wastes are becoming prevalent in the oceans and the most common type of debris, bacterial communities in the aquatic environment that can degrade specific polymers such as Polyethylene terephthalate (PET) and Polyethylene (PE) are also warranted. Another paper tackled the idea of a PET-degrading bacteria (Lee *et al.*, 2021), where multiple PET-degrading bacterial

strains, namely *T. fusca*, *I. sakaiensis*, and *P. mendocina* may be considered to develop an industrial co-culture solution. Accordingly, producing a co-culture solution would present a sustainable method to biodegrade plastics and may help retain the plastic's material value. The two publications mentioned above could be used as the basis for further research where the methodology outlined (Gao & Sun, 2021) and the foundation for the feasibility of applying a solution to a broader scale to improve the efficiency and coverage of plastic-degrading bacteria (Lee et al., 2021).

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