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Plastic-eating Bacteria as a Remedy for Plastic Pollution

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Abstract
Plastic pollution can have negative effects on human health as well as serious effects on marine ecosystems. The widespread production of polyethylene terephthalate (PET) single-use plastics poses a significant threat to aquatic and terrestrial ecosystems in terms of plastic waste. PET is a strong, clear and light plastic that is typically used for food and beverage packaging, as well as for other single-use applications. As a result, removing plastic from the environment is not only difficult but also ineffective financially. Numerous strains of bacteria are capable of biodegrading a variety of plastics. Utilizing beneficial micro-organisms that are capable of breaking down plastic could be an effective and long-term solution to all of the problems. *Ideonella sakaeinsis* 201-F6 is the most well-known heterotrophic bacteria that can use PET as its primary source of energy and carbon to degrade plastic in the environment. It has a place with the sort of *Ideonella* and the family Comamonadaceae. With the assistance of specific enzymes like PETase and MHETase, it can ultimately degrade plastic, potentially reducing the problem of plastic waste. Polyethylene terephthalate (PET) is first transformed by the PETase into mono-(2-hydroxyethyl) terephthalate (MHET), after which MHET is hydrolyzed to produce ethylene glycol (EG) and terephthalic acid (TPA). *I. sakaiensis* offers a novel strategy for recycling PET because it can mediate the direct transformation of non-biodegradable PET into plastic that is better for the environment.

Keywords: Plastic; Microplastic; Pollution; Bacteria; Remedy; Ecosystems; Environmental health

Introduction

Plastics are extremely useful materials that have significantly altered our society. However, plastic pollution has grown in both natural and man-made environments as a result of the widespread use (Singh, 2022). Plastic is inexpensive, durable, lightweight, strong and impervious and has been referred to as an uncooperative polymer. The drawn-out gathering of plastic, especially the bioplastic, causes numerous well-being and natural dangers (Ahmed, 2022; Tayal et al., 2023; Singh and Singh, 2024; Kaur et al., 2023; Gupta et al., 2022). The development of these polymers in water, horticultural soil, and residue has raised many worries all around the world. The total quantity of plastics that is produced every year has been estimated at around 140 million tons, which is eventually made accessible to the ecosystem as industrial waste products (Adetunji and Anthony, 2021). Its stability and durability have made it an essential part of our lives. Despite its superiority over different materials, it poses a main threat to the surroundings because long-chain polymeric molecules take heaps of years to decompose naturally (Bakht et al., 2020). Among all plastics, Polyethylene terephthalate (PET) is the most beneficent polyester synthetic material internationally and has been significantly used for packaging, clothing and beverage bottles. PET is made of terephthalic acid (TPA) and ethylene glycol (EG), which might be polymerized via ester linkage (Seo et al., 2018). Its durability, transparency, and low permeability to gases make PET an appealing product for the packaging marketplace and clothing industry. At the same time, excessive portions of PET were launched into the surroundings at some stage in the system of its application, production, and disposal. Although production of this polymer is insignificant, because of the chemical inertness of its fragrant compounds, it will become adverse at some stage in the recycling process, making it cost-powerful and energy-consuming (Fecker et al., 2018). It is envisioned that it takes masses of years for the whole degradation of PET plastics with the aid of using micro-organisms inside the environment. Now, the collection of PET waste is continuously rising and starting to threaten ecosystems across the globe (Soong et al., 2022). Although synthetic polymers and PET plastics are considered non-toxic, micro granules and large particles thereof are durable, ubiquitous in marine or terrestrial habitats, and hoard in living organisms. Often, they are also the bearers of potentially toxic additives and colorants. Plastics readily contaminate marine ecosystems and has resulted in their penetration into the animal and human food chains, which has been linked to various adverse health effects, including immune disorders and congenital disabilities, as well as cancer (Palm et al., 2019).

Numerous micro-organisms have been reported for their biodegradative potential for plastics. Some of these strains include *Aspergillus*, *Streptococcus*, *Bacillus*, *Penicillium*, *Staphylococcus*, *Pseudomonas*, *Streptomyces* and *Moraxella* (Adentunji and Anthony, 2021). Recently, the strain of the micro-organism *Ideonella sakaiensis* 201-F6 was discovered, which grows on low-crystallinity PET films (Palm et al., 2019). This bacterium incorporates particular enzymes including mono (2-hydroxyethyl) terephthalic acid hydrolase (MHETase) and PET hydrolase (PETase) to hydrolyze PET into ethylene glycol (EG) and terephthalic acid (TPA) monomers, that are then digested further. These enzymes have drawn international interest as they may be used for the bioconversion of PET. For constructing PETase and MHETase strains, a targeted gene disruption system has been developed in *Ideonella*. Disrupting growth revealed that for the degradation of PET, PETase is the single enzyme for degradation in the bacteria, while MHETase and PETase play important roles in the assimilation of PET. *Ideonella sakaiensis* is a rod-shaped, non-spore-forming, gram-negative, rod-shaped, aerobic bacterium that belongs to the class Betaproteobacteria. The optimal growth conditions include the optimal pH (7 to 7.5) and temperature (30 to 37°C) (Hachisuka et al., 2021). As *I. sakaiensis* grows only under mild conditions, the stability of *I. sakaiensis* PETase (IsPETase) is relatively low (Joo et al., 2019).

Enzymes Involved in PET Degradation

More than 24 different enzymes have been identified with PET-degrading abilities. All of those enzymes are hydrolases, catalyzing the degrading of the PET polymer into TPA, EG, Bis (2-hydroxyethyl) terephthalate (BHET) and MHET (Cunha et al., 2021). Microbes can degrade plastics through attachment to the surface of the plastic via enzymatic hydrolysis (Seo et al., 2018).

Ideonella sakaiensis PETase (IsPETase)

The IsPETase is produced via a bacterium, *Ideonella sakaiensis* 201-F6, that can assimilate PET as a main electricity and carbon source (Cunha et al., 2021). The intriguing capability of IsPETase for the degradation of PET has been receiving much attention (Seo et al., 2018). IsPETase may be an extra environmentally pleasant and green opportunity for the chemical healing of PET. It is the mesophilic enzyme that has a lower optimal reaction temperature (between 20°C and 40°C) than the glass transition temperature of PET. With the enzymatic breakdown, amorphous polymers can be retrieved (Brott et al., 2021). This enzyme was confirmed among five and a hundred and twenty instances of better depolymerization pastime in opposition to PET films at 30°C (Fecker et al.,2018). IsPETase showed a higher specificity for PET. The enzyme reveals poor durability as much of its activity would be lost within 12 h of inoculation at 37°C.

Structure of IsPETase

IsPETase incorporates 290 amino acid residues, which exist as a purposeful monomer, and with numerous structural, experimental and computational studies, the three-dimensional structure has been explored (Cunha et al., 2021). First, IsPETase is part of α/β hydrolase superfamily, and the central twisted β-sheet is made up of nine mixed β-strands (β1-β9) enclosed by seven α-helices (α1-α7). Remarkably, the peculiar shape of the β6 strands in IsPETase prevents the central β sheet from functioning properly (Joo et al., 2019). On the energetic site of this enzyme is the conserved serine hydrolase Gly-x1-Se-x2-Gly motif (Gly158-Trp159-Ser160-Met161-Gly162). Because the pET15b vector was used, the recombinant IsPETase protein also contains amino acid residues at both the N and C termini (Met13-Met33 and Leu291-Gln312). Furthermore, residues Ser31-Gln292 are present (Seo et al., 2018).

Mechanisms of biodegradation

Polyethylene terephthalate

Plastic degradation by microbial and/or enzymatic means is a favorable strategy to depolymerize waste petro-plastics into monomers for recycling or mineralize them into carbon dioxide, new biomass, and water with consequent production of higher-value bioproducts. Excretion of extracellular enzymes involved during the biodegradation of plastics by a microorganism, incorporation of the enzyme to the plastic surface, and breakdown to short polymer intermediates, which are eventually assimilated by microbial cells as the source of carbon to release CO_2 (Montazer et al., 2020). Microbes can degrade highly crystalline polymers; however, their application is restricted to commercial plastics. Microbiomes interact with abiotic factors to change the polymer structures (Tamoor et al., 2021). Microorganisms initially attach to the PET film surface during its disintegration and then they create extracellular PET hydrolases that bond to the PET film even more and begin the process of biodegradation. PET hydrolases exploit the ester bond of PET, which then hydrolyzes it into Ethylene Glycol (EG) and Terephthalic Acid (TPA) and generate incomplete hydrolysis products, such as MHET and BHET. MHET can be hydrolsed by MHETase into TPA and EG. PET hydrolases can further break BHET to produce MHET, EG and TPA. The TPA and EG products can be utilized by different microorganisms and then metabolized into the tricarboxylic acid cycle (Yuan et al., 2021). Crystallinity of PET, pH, temperature of the hydrolysis reactions, buffer strength and the nature of additives/substituents present in plastics (as plasticizers) are some of the factors affecting the enzymatic degradation of PET (Maurya et al., 2020).

Polyethylene

Polythene or polyethylene is a polymer of ethylene gas that is commonly used in our day-to-day life like grocery bags, shampoo bottles, bulletproof vests, etc. Several kinds of polythenes include linear HDPE (high-density polyethylene), branched LDPE (low-density polyethylene), and LLDPE (linear low-density polyethylene) (Khanam and AlMaadeed, 2015; Chandra et al., 2015). The majority of bacterial strains can damage PE's surface and create biofilms. Because of their special capacity to break down and absorb polymers by extracellular hydrolytic and oxidative enzyme activities, *Pseudomonas sp*. facilitate the uptake and degradation of the fragments of polymers and control the interaction between biofilms and polymer surfaces (Ghatge et al., 2015). PET can naturally degrade by thermal oxidation, but in ambient settings, photo-oxidation brought on by UV light and hydrolytic cleavage are frequent (Chamas et al., 2020).

Table 1. Comparison between main types of polyethylene

Type	**LDPE**	**LLDPE**	**HDPE**
Structure	Short chain branch (SCB)+ Long chain branch	The high degree ofshort branching	Linear or low degreeof short branching
Density	0.917-0.924g/cm^3	0.915-0.95g/cm^3	0.941 - 0.965 g/cm^3
Crystallinity	Low crystallinity (less than 50-60%)	Semi-crystalline (40-55%)	The high degree of crystallinity (70-80%)
Characteristics	Semi-rigid, Translucent Good chemical resistance, Weather proof, very tough	Very flexible with high-impact strength, Translucent, Good stress crack and impact resistance, excellent for mild and strong buffers.	Flexible, Waxy, Tough, Good at low temperature, Easy to process by most methods.
General applications	• In dispensingbottles • Tubing • Plastic parts of computer components • various molded laboratory equipment	• used as plastic wrap • pouches • stretch wrap • covering of cables • in lids and pipes	• shampoo bottles • toys • chemical containers • milk jugs • pipe systems
Recycling code	4 LDPE	4 LDPE	2 HDPE

Polyvinyl chloride

In its rigid or plasticized form, poly(vinyl chloride) (PVC) is a synthetic polymeric substance that has been extensively utilized for many years. Low molecular weight polyvinyl chloride could be biodegraded by white rot fungus (Basidiomycetes) under conditions of nutrient limitation including nitrogen, sulphur or carbon (Montazer et al., 2020). The predominant plasticizers employed for PVC plastics include phthalic esters, carboxylic acid epoxides, esters, polyesters, and renewable resource-based plasticizers. Many metabolic genes and enzymes in the microorganisms are necessary for the assimilation of phthalates (Mucha et al., 2022). *Pseudomonas citronellolis* and *Bacillus flexus* are found to be PVC film biodegraders (Giacomucci et al., 2019). The biodegradation of phthalates involves subsequent hydrolysis of the ester linkage and phthalic acid (Mucha et al., 2022). Degradation of solid polymers like PVC by the microbes requires the formation of biofilm. The microbes use these insoluble substrates through activities of enzymes (chitinase and glucanase). The development of such biofilms on the surface of synthetic polymers can prove to be a very effective method for the degradation of these polymers in vitro (Manderia et al., 2022).

Polystyrene

Polystyrene, the third most foremost petroleum-based plastic is used for packaging containers, insulating materials and disposable cups. It contains about 7% of the entire amount of plastics produced. Due to its long persistence in the environment, polystyrene waste biodegradation efficiency is very low in natural ecosystems and is attributable to serious environmental pollution (Jiang et al., 2021). The degradation can start either from the cleavage of the side chain or the main chain, which leads to different degradation pathways. Styrene is biodegraded by styrene monooxygenase (SMO) into styrene epoxide and then by styrene oxide isomerase into 4-maleylacetoacetate, phenylacetaldehyde dehydrogenase (PAALDH), phenylacetate hydroxylase (PAAH), 2- hydroxy phenylacetate hydroxylase (HPAAH) and homogentisate 1,2-dioxygenase (HGADO). 4-Maleylacetoacetate is subsequently transformed into acetyl-CoA via the beta-oxidation pathway, which is followed by the TCA cycle to the central biosynthetic pathways (Zhang et al., 2022).

Table 2. Various literature reports on plastic biodegradation by thermophiles

Sr. no.	Polymer type	Micro-organism	Isolation source	Temp.	Effectiveness of degradation	References
1.	Polyethylene (PE)	*Brevibacillus borstelensis* strain-707	Soil	50°C	11% after 30days.	Hadad etal. (2005)
2.	Polyethylene terephthalate (PET)	*Thermobifida fusca*	Soil	55°C	50% decreasein average mol. weight ofpolymer for 3 weeks	Atanasova et al. (2021)
3.	Polyethylene bags	Pseudomonassp. Aspergillus glaucus	Mangrovesoil	42°C	20.58% and 28.80% weight loss	Zhang etal. (2022)
4.	Polystyrene (PS)	*Massilia sp.* FS1903	The gut of larvae of *Galleria melonella*	30°C	The weight of PS film significantly with 12.97 ± 1.05% weight loss.	Jiang et al. (2021)
5.	Polyviny lchloride (PVC)	*Klebsiella sp.* EMBL-1	Gut of insect larvae of *Spodoptera fungiperda*	30°C	The final average weight loss ofPVC film is 19.57% after 90 days	Peng et al. (2022)
6.	LDPE	*Pseudomonas citronellolis*	Municipal landfill	37°C	17.8% weightloss	Zhang etal. (2022)
7.	*Fusarium redolens*	Garden soil	LDPE and HDPE labeled with ^{14}C, 100 days	Not specified	$^{14}CO_2$ liberation	Zhang etal. (2022)

Conclusion

Pollution is a worldwide phenomenon and no continent is immune to its negative environmental impact. Plastic-degrading bacteria point to the unexploited potential of bacteria and their enzymes in curbing and reducing plastic pollution in different natural environments. There is a broad range of degradation pathways employed by a large biodiversity of micro-organisms to metabolize plastics. Numerous micro-organisms including *Aspergillus*, *Streptococcus*, *Bacillus*, *Penicillium*, *Staphylococcus*, *Pseudomonas*, *Streptomyces* and *Moraxella* have been reported for their biodegradative potential for plastics. Such organisms are the main focus of research for the clean-up of environmental pollution. *Ideonella sakaiensis* has been found to have enormous potential to figure out the plastic waste problem due to their ability to degrade polyethylene terephthalate. With the assistance of specific enzymes like PETase and MHETase, it can ultimately degrade plastic, potentially reducing the problem of plastic waste. This breakdown has enabled the development of novel biological systems capable of degrading PET at unprecedented rates, offering a promising solution to persistent plastic pollution problem. It is expected to become an optimal environmentally friendly bio-solution that can be carried out sustainably.

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SK, ZS and BS conceived the concept, wrote and approved the manuscript.

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Competing interest

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Ethics approval

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