A Comprehensive Review on the Diverse Arsenal of PET-Degrading Organisms

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ABSTRACT

Polyethylene terephthalate (PET) is a common and persistent plastic that accumulates in the environment and causes serious ecological problems. It is widely used for packaging, textiles, and other applications, but its recycling rate is low and its degradation rate is slow. Therefore, PET waste poses a threat to the natural ecosystems and human health, as it can release toxic chemicals, disrupt the food chain, and affect the biodiversity. To mitigate this issue, an emerging and promising strategy is to use microorganisms that can biodegrade PET into reusable monomers. These monomers can be further processed into biodegradable polymers or other products, thus reducing the environmental impact of PET. This paper provides a comprehensive review of the diversity and efficacy of various biodegradation agents, including bacteria, fungi, algae and wax worms for PET. It also discusses the enzymatic mechanisms and influencing factors of PET degradation by these biological entities. Furthermore, the paper emphasizes the practical applications of employing these biodegradation agents in waste management and bioremediation strategies. It underscores their potential to transform PET waste into valuable resources. The review serves as an up-to-date and comprehensive guide to the microbial degradation of PET and offers insights and directions for future research and applications.recycling rate is low and its degradation rate is slow. Therefore, PET waste poses a threat to the natural ecosystems and human health, as it can release toxic chemicals, disrupt the food chain, and affect the biodiversity. To mitigate this issue, an emerging and promising strategy is to use microorganisms that can biodegrade PET into reusable monomers. These monomers can be further processed into biodegradable polymers or other products, thus reducing the environmental impact of PET. This paper provides a comprehensive review of the diversity and efficacy of various biodegradation agents, including bacteria, fungi, algae and wax worms for PET. It also discusses the enzymatic mechanisms and influencing factors of PET degradation by these biological entities. Furthermore, the paper emphasizes the practical applications of employing these biodegradation agents in waste management and bioremediation strategies. It underscores their potential to transform PET waste into valuable resources. The review serves as an up-to-date and comprehensive guide to the microbial degradation of PET and offers insights and directions for future research and applications.

Keywords: *Biodegradation, Cutinase, Microplastic, PET, Sustainability.*

1. Introduction

PET plastic, or polyethylene terephthalate, is a commonly used synthetic polymer in the packaging industry because of its versatility, durability and recyclability. It is commonly found in various forms, such as bottles, containers, and films, and is recognized by its resin identification code. PET plastic is made up of a series of monomers, specifically, ethylene glycol (EG) and terephthalic acid (TPA), resulting in a clear, transparent and lightweight material. The environmental impact of PET plastic, however, has raised concerns about its contribution to plastic pollution and microplastics in the environment (Das et al., 2021; Kaur et al., 2023).

The low manufacturing expenses of plastic generated from fossil fuels and the material's exceptional durability are significant benefits, however, they have become a burden for the natural world (Bollinger et al., 2020). Plastic debris is being accumulated at a significantly greater rate than it is being recycled which is a major cause of concern. The existing plastic load on the environment has ignited the need for research on biological alternatives to plastic degradation which can be sustainably commercialized in order to minimize the plastic and the microplastic pollution

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which eventually evade easily in the water bodies, causing a threat to marine ecosystems (Kaur et al., 2023).

This article aims to explore various aspects related to the natural degradation of polyethylene terephthalate (PET) by microbes. Firstly, it delves into the diverse types of microbes that possess the capability to naturally degrade PET. Secondly, it assesses the efficiency of PET degradation exhibited by these microbial agents. The article then delves into the underlying mechanisms and enzymatic actions involved in the degradation processes, shedding light on the intricate biochemical processes at play. Finally, it discusses the potential implications and expectations for the future of this approach, offering insights into the evolving landscape of PET degradation by microbes in the near future.

As per the studies conducted so far, it has been found that a variety of microbes such as bacteria, fungi, algae and some species of wax-worms are capable of metabolising PET plastic naturally, thereby, resulting in its breakdown to simpler monomeric forms **(Table 1)**.

Table 1: *Outlines some of the various species of microbes which break down PET plastic.*

PET-PLASTIC DEGRADING BACTERIA				
BACTERIA	**ENZYME INVOLVED**	**SUBSTRATE USED**	**PRODUCT FORMED**	**REFERENCE**
Thermobifida fusca	TfCut1 & TfCut2 (Cutinase)	PET	MHET, BHET & TPA	Roth et al., 2014; Then et al., 2015; Barth et al., 2015; Müller et al., 2005
Thermobifida cellulosilytica	Thc_Cut1 & Thc_Cut2 (Cutinase)	PET, 3PET	MHET & TPA	ArnlingBååth et al., 2022; Gamerith et al., 2017; Ribitsch et al., 2017; Herrero Acero et al., 2011
Thermobifida alba	Tha_Cut1 (Cutinase)	PET	MHET & TPA	Ribitsch et al., 2012; Kitadokoro, et al., 2012
Clostridium botulinum	Cbout_EstA, Cbout_EstB (Esterase)	PET	MHET & TPA	Biundo et al., 2017; Perz et al., 2016
Pseudomonas sp.	PE-H (Hydrolase)	PET	TPA & EG	Bollinger et al., 2020
Ideonella sakaiensis	PETase & MHETase	PET	TPA & EG	Yoshida et al., 2016; Kaur et al., 2023
Bacillus sp.	BsEstB (Esterase)	PET	MHET & TPA	Samak et al., 2020
Saccharamonospora viridis	Cut190 (Cutinase)	PET	TPA	Numoto et al., 2018; Kawai et al., 2014
Thermonospora curvata	Polyester hydrolase	PET	MHET, BHET, TPA & EG	Kim et al., 2022 (a)
PET-PLASTIC DEGRADING FUNGI				
FUNGI	**ENZYME INVOLVED**	**SUBSTRATE USED**	**PRODUCT FORMED**	**REFERENCE**
Aspergillus tamarii	Lipase & Cutinase	PET	TPA	Anbalagan et al., 2021
Penicillium simplicissimum	Lipase	(PC)-PET	TPA, MHET, BHET	Moyses et al., 2021
Penicillium citrinum	Polyesterase	PET	TPA, MHET, BHET, BA	Ahmaditabatabaei et al., 2021
Pleurotus spp.	Hydrolase	PET	MHET, BHET, TPA, EG	Odigbo et al., 2023
Fusarium solani/oxysporum	Cutinase	PET	TPA, BHET, MHET	O'Neill et al., 2007
Humicola insolens	Cutinase	PET	TPA, MHET, BHET	Carniel et al., 2017

The traditional physical and chemical methods of plastic degradation present an array of drawbacks which could prove to be lethal in the coming future; therefore, the employment of different microbial enzymes provides a much cleaner and environment-friendly approach to plastic bioremediation **(Figure 1)**. To completely eliminate plastic polymers by biological means, the polymer must first be broken down into simpler oligomeric chains and eventually monomers that can diffuse across the plasma membrane, subsequently undergoing absorption and eventual intracellular metabolism. However, the mode of PET degradation employed by these microorganisms differ in terms of its efficiency, intermediate formation, reaction rate, enzymes involved, amount and type of product formed, viability and the optimum conditions required. While it has been found that a greater species of bacteria can metabolise PET plastic, research is going on to discover new species of different microbes with better plastic degrading efficiency.

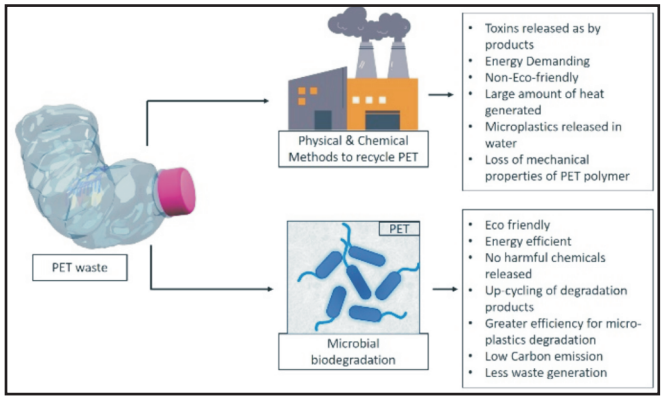

Figure 1: *Comparison between mechanical, chemical and biological ways of plastic degradation.*

2. Bacteria to the Rescue

2.1 *Thermobifida fusca*

Thermobifida fusca, previously known as *Thermomonaspora fusca*, is classified as a moderate thermophilic soil bacterium, displaying optimal growth at a temperature of 55°C. Notably, its robust enzymatic array includes cellulases, among other extracellular enzymes, recognized for their impressive stability at elevated temperatures and a wide pH spectrum spanning from 4 to 10. *T. fusca* exhibits the capacity to degrade various plant cell wall components except for lignin and pectin, showcasing its versatility in breaking down complex polysaccharides. This actinomycete microbe efficiently metabolizes diverse simple sugars and carboxylic acids, contributing to its adaptability and growth across various substrates (Zainudin et al., 2019).

A type of hydrolase from *T. fusca*, TfH was the very first enzyme discovered to be capable of decomposing PET in 2005 (Müller et al., 2005). *Thermobifida fusca* KW3 is known to produce two pole ester hydrolases viz. TfCut1 and TfCut2. The enzymes show nearly 82% and 83% sequence homology respectively with the previously isolated strain of Est119 – an esterase isolated from *T. alba* (Then et al., 2015). TfCut2 hydrolyzes PET into monomers: Mono-(2-hydroxyethyl) terephthalic acid (MHET), which forms the major chunk of the yield (about 75%) of the products while TPA and bis-(2-hydroxyethyl) terephthalate (BHET) have a comparatively smaller yield (about 25%). Barth et al., 2015 reported a reaction time of about an hour for hydrolysis of 1g of PET resulting in the production of about 0.55g of MHET along with smaller amounts of BHET and TPA. In comparison with such a rapid degradation process, it took significantly longer to convert the intermediates MHET and BHET into TPA. Thus, these intermediates (MHET and BHET) were found to hinder the enzymatic activity of TfCut2 due to the occupancy of the enzyme's active site by the ester bonds of these intermediates. TPA and ethylene glycol (EG), however, caused no inhibition of the enzymatic activity (Barth et al., 2015).

In contrast to PET degradation, the enzymes however, showed negligible results when treated with PHB and polybutylene terephthalate (PBT), which is most probably due to their high melting point and crystalline nature. This in turn indicates that the enzyme has a specialised structure that aids in the degradation of aromatic polyesters by attacking their ester bonds (Muller et al., 2005). However, TfCut2 loses its enzymatic activity by 100% within an hour at an incubation temperature of 65.6°C and can therefore not be engaged in PET hydrolysis at its glass transition temperature which is about 7°C (Then et al., 2015).

The enzyme belongs to the α/ß-hydrolase fold family, with a central ß-sheet of nine strands surrounded by 11 α helices on both sides. The active site consists of S130, D176 and H208, which form a catalytic triad at the bottom of the enzyme structure. The enzyme is highly thermostable, which enhances its efficiency (Roth et al., 2014). Then et al., 2015 observed that the presence of Ca^{2+} and Mg^{2+} ions affected the PET degradation activity of the enzyme. Compared to other polyester hydrolases, TfCut1 and TfCut2 had a 2.5-fold reduction in PET hydrolysis in the presence of Ca^{2+} ions at 55°C and a 1.8 to 2.6 fold reduction at 60°C. On the other hand, the addition of Mg^{2+} ions caused an increase in the activity at the same temperatures. Thus, the thermostability and the efficiency of the enzyme can be improved by replacing Ca^{2+} ions at binding sites with positive ions like arginine (Then et al., 2015).

However, Haugwitz et al. stated that *T. fusca* is not able to efficiently metabolize products of PET breakdown, such as MHET. Since *T. fusca* is unlikely to be able to translocate MHET in the cytosol, the function of one of its

carboxylesterases called TfCa, in PET breakdown in a natural setting is ambiguous (von Haugwitz et al. 2022). Though plastic bottles with high transparency are being manufactured, PET with low crystallinity should be preferred, so as to increase the chances of microbial degradation.

2.2 *Thermobifida cellulosilytica*

T. cellulosilytica is a member of the Actinomycetota phylum, Actinomycetes class, Streptosporangiales order, Nocardiopsidaceae family, and *Thermobifida* genus. It is a thermophilic bacterium, isolated from composted manure that had been warmed. It may be cultivated at 45℃ on Czapek Peptone Agar (DSMZ Medium 83) (ArnlingBååth et al., 2022).

Various polymers can be hydrolyzed by enzymes from the cutinase family. Two such enzymes, Thc_Cut1 and Thc_Cut2, are produced by *T. cellulosilytica* and differ by only 18 amino acids (ArnlingBååth et al., 2022; Gamerith et al., 2017). However, they have structural and functional differences. Thc_Cut1 has a kcat value that is 100 times higher than Thc_Cut2. Thc_Cut1 also releases more MHET, TPA, BA and HEB than Thc_Cut2 when they react with the model substrate 3PET (Herrero Acero et al., 2011). Thc_Cut1 mainly produces TPA and MHET from PET, while Thc_Cut2 mainly produces MHET and no BHET. Thc_Cut1 and Thc_Cut2 have different activity on PLLA, which may be related to their structural differences, i.e., Thc_Cut2 has a longer and more hydrophobic region than Thc_Cut1 (Ribitsch et al., 2017).

The two variants, Thc_Cut1_koAsn and Thc_Cut1_koST have been synthesised by glycosylation site knockout; no significant difference in the extracellular synthesis of the enzymes have been found. TPA and MHET were detected via high-performance liquid chromatography when the enzyme acts on PET, for the native cutinase and the mutants. The native cutinase, Thc_Cut1 when acted on polybutylene carbonate (PBC) can reduce up to 93% of its mass. The enzyme when expressed in E. coli shows no glycosylation, at the same time, when expressed in P. pastoris it leads to glycosylation, which in turn increases the stability of the enzyme (Gamerith et al., 2017).

Therefore, *T. cellulosilytica* is a potential PET degrader due to its ability to produce enzymes capable of breaking down PET. Research suggests that this microorganism's enzymatic activity can contribute to the degradation of PET, making it a promising candidate for bioremediation efforts aimed at reducing PET waste in the environment.

2.3 *Thermobifida alba*

T. alba is a thermophilic bacterium belonging to the class Actinobacteria and family Nocardiopsaceae which can be extracted from compost (Kitadokoro et al., 2019). It is an aerobic, gram-positive bacterium that is a member of the *Thermobifida* genus (Shivlata and Satyanarayana, 2015). The strain of T. alba AHK119 can be cultivated in Luria-Bertani medium (LB medium) at about 50℃ with constant shaking for about three days. (Thumarat et al., 2015)

A cutinase isolated from *T. alba*, Tha_Cut1 and is found to be similar to a cutinase from its close relative *T. cellulosilytica*. Esterases, lipases and cutinases are the enzymes majorly involved in PET hydrolysis. Although not all cutinases can degrade polyester-type plastics, current research has revealed that some cutinases play critical roles in the degradation of polyester. Tha_Cut1 however showed better enzyme-substrate interaction owing to its slight change in primary structure. Tha_Cut1 differs from Thc_Cut1 in having four different amino acids outside the active site which is probably a reason for its better efficiency. Furthermore, their 3D protein structure lacks a lid over the active site, a trait that is prevalent in lipases and is required for interfacial activation. *Thermobifida* has a number of cutinases that have been cloned and identified as polyester-degrading enzymes. Est119 is a cutinase that degrades plastic and has broad substrate selectivity for polyesters as well, it is expressed by est119 gene from *Thermobifida alba* (Ribitsch et al., 2012; Kitadokoro et al., 2012).

Certain cutinases have also been isolated from fungal species like *Aspergillus oryzae, Penicillium citrinum* and *Fusarium solani*, however, cutinases from *Thermobifida* species exhibit superior qualities with respect to their wide optimum temperature range, which not only increases the reaction rate but also favours enhanced substrate binding (Ribitsch et al., 2012).

The enzyme is a carboxylesterase, similar to the serine hydrolases having a catalytic triad consisting of Ser-His-Asp. It consists of four calcium binding sites and the whole molecule resembles the shape of a ladybug, wherein the active side groove is placed adjacent to the belly-like side of the bug, at the top resembling its head. This kind of structure helps the enzyme bind firmly with the substrate molecule. The motion of cutinase running over the PET surface, digesting it side by side coincides with the walking gesture of the beetle (Kitadokoro et al., 2019).

The crystallisation of the enzyme molecule reveals its similarity with α/β-hydrolase comprising of a central β-sheet (9 β-pleats) arranged in order 1-2-4-3-5-6-7-8-9 flanked by about 9 α-helices on both sides of the sheet (1,2,7,8,9 on one side and 3,4,5,6 on the other side) (Kitadokoro et al., 2012).

The catalytic triad is situated among the loops of β strands and α helices, along with an oxyanion hole formed by the main chain amides of Met and Tyr. The C-terminal region is stabilized by disulfide bonds. A polyethylene glycol moiety is attached to the interface of the refined model's asymmetric unit, which consists of two monomers that create a dimer interface. A possible glycol-binding site on

the protein is suggested by the presence of a polyethylene glycol-binding site. A potential polymer-recognizing groove can be observed through the catalytic pocket. The groove is characterized by water molecules that connect to hydrophilic amino acids along the groove, forming an alternating pattern of polar and nonpolar residues (Kitadokoro et al., 2012; Thumarat et al., 2015).

Thermobifida alba exhibits potential as a PET plastic degrader due to its enzymatic activity yielding TPA and MHET. However, further studies and advancements are required to enhance its properties and industrialise its use for bioremediation and waste management strategies (Haugwitz et al., 2022).

2.4 *Pseudomonas* spp.

Pseudomonas spp. are a group of gram-negative, rod-shaped, and motile bacteria which grow in a wide range of environments. They are typically aerobic and prefer temperatures between 25°C and 37°C. They can grow in a variety of media, including nutrient agar and Luria-Bertani (LB) broth. Depending on its species and strain, the particular growing requirements may, however, differ (LaBauve and Wargo, 2012).

Pseudomonas species from environmental samples have been identified as PET plastic degraders, and the genus *Pseudomonas* is among the most frequently reported degraders with varying efficiencies for a broad range of plastic polymers (Wilkes and Aristilde, 2017). An unspecified *Pseudomonas* sp. strain exhibited limited capability to degrade PET, as observed in previous studies (Müller et al., 2005; Jun et al., 1994). Nonetheless, an impactful breakthrough emerged with a cutinase derived from *P. mendocina*, displaying remarkable efficacy in breaking down PET with low crystallinity. This enzyme led to a notable 5% reduction in film weight over 96 hours, concurrently generating valuable TPA and EG as exclusive byproducts. The cutinase from *P. mendocina* hydrolysed PET by initiating a consequential pathway, enabling TPA and EG assimilation into the bacterial cell's intracellular metabolism, potentially offering a promising route for enhanced PET degradation (Ronkvist et al. 2009; Wilkes and Aristilde, 2017). A study by Badahit, 2018, discovered that *Pseudomonas* spp. decomposed 7.6% and 8.2% of plastic over a month at 30°C and 37°C, respectively (Badahit, 2018).

In *P. putida*, the genetic machinery governing EG metabolism has been extensively elucidated. Strain JM37 on the other hand efficiently uses EG as the only source of carbon and energy, KT2440 displays limited growth despite EG utilization. EG is enzymatically converted to glyoxylate, generating reducing equivalents like PQQH2 or NADH (Mückschel et al., 2012; Wehrmann et al., 2017). The glyoxylate shunt efficiently converts glyoxylate into CO_2 and reduced equivalents. In *P. putida* and *P. aeruginosa*, PedE, PedH, and ExaA enzymes, pivotal for ethanol utilization, also play crucial roles in EG metabolism, enhancing effective degradation (Mohanan et al., 2020).

The common α/β hydrolase fold is a characteristic feature of lipases from *Pseudomonas* spp. that degrade PET. These lipases have a Gly-X1-Ser-X2-Gly sequence and a catalytic triad (Ser-His-Asp) that is essential for their activity. The triad consists of Ser as the nucleophilic agent, His as the basic element, and Asp as the acidic component. A lid-like peptide chain conceals the active site, controlling its exposure and influencing oxyanion hole formation during substrate cleavage. This lid presence can hinder substrate access and hydrolysis. Lipases also necessitate interfacial activation. While certain lipase family members can break down PET fibres and PET films remain challenging due to restricted substrate access (Gao et al., 2021).

Recently, *P. aestusnigri*, a marine bacterium, exhibited polyester degradation through its newly identified enzyme, PE-H, classified as a type IIa PET hydrolase. The crystal structure of PE-H was unveiled, showing similarity to cutinases and other PET hydrolytic enzymes. *P. aestusnigri* demonstrated hydrolytic activity on various polyester substrates, except for commercial PET film. The canonical α/β-fold of PE-H harbours catalytic residues forming the triad, with structural modifications that expand the active site cavity. PETa and BHET were successfully degraded into MHET by PE-H, suggesting its potential for PET degradation (Bollinger et al., 2020).

2.5 *Ideonella sakaiensis*

Ideonella sakaiensis, also known as the first plastic-eating bacteria, was discovered in March 2016 from the sludge outside a bottle recycling plant in Osaka (Yoshida et al., 2016). This bacterium can break down PET plastic organically by using two enzymes, *Is*PETase (PET hydrolase) and MHETase (MHET hydrolase), that cleave PET polymers into simple monomers such as TPA and EG, with BHET and MHET as intermediates. This bacterium has a great plastic degrading efficiency and holds tremendous potential for future commercialization (Kaur et al., 2023).

2.6 *Saccharomonospora viridis*

Saccharomonospora viridis is a thermophilic, generally gram-negative, aerobic bacteria that grows best at a temperature of 55°C, a temperature of 45°C is optimum for the creation of aerial mycelium and the generation of pigment (Pati et al., 2009).

It possesses a cutinase-like enzyme with strong PET degradation activity. It has been demonstrated that Cut190_S226P/R228S, a mutant of this enzyme known as Cut190*, has great thermal stability and strong PET-degrading activity (Hantani et al., 2018).

A novel enzyme, Cut190, which is a cutinase-type polymerase, has been isolated from *S. viridis* AHK109 that has been found to degrade PET. A unique feature of it is that Ca^{2+} binding regulates function and stability, indicating that Cut190 is a novel Ca^{2+} activated cutinase. Three calcium ion binding sites are found on Cut190S176A, a mutant of the wild-type enzyme Cut190 (Numoto et al., 2018; Maurya et al., 2020 (a)). According to Kawai et al., the wild type enzyme has the potential to hydrophilize PET film at 50℃, overnight with a reaction mixture containing TPA but no BHT at the temperature of 50℃ and 65℃. The recombinant Cut190 (S2226P/R228S) shows maximum activity between pH 6.5-7.0 and temperature 65℃. It shows decent activity of 76.3% even at temperatures as high as 70℃, however the activity decreases drastically once it reaches a temperature of 75℃. (Kawai et al., 2014). Therefore, proper future developments could help in improving its efficiency which could further aid in plastic degradation on a larger scale.

2.7 *Thermomonospora curvata*

Thermomonospora curvata belongs to the family Thermomonosporaceae and is a gram-positive, chemoorganotrophic microorganism capable of facultative aerobic growth (Chertkov et al., 2011).

Operating as a thermophilic actinomycete, it exhibits notable proficiency in breaking down synthetic polyesters, particularly PET (Wei et al., 2014). Analysis *of T. curvata* DSM43183's genome has unveiled two distinct genes, Tcur1278 and Tcur0390, encoding potential enzymes for polyester hydrolysis. Remarkably, Tcur0390 has exhibited enhanced hydrolytic effectiveness against PET nanoparticles, especially within the temperature range of up to 50℃ (Kawai et al., 2019). Similarly, Tcur1278 showcases its own enzymatic activity against PET nanoparticles, albeit at elevated temperatures of 55℃ and 60℃. These findings significantly underscore the relevance *of T. curvata* in the realm of PET degradation, accentuating the enzyme-specific intricacies tied to temperature-induced efficiency. Such insights hold the potential to play a pivotal role in shaping strategies for effective biodegradation of PET materials (Wei et al., 2014).

2.8 Other Bacteria

Besides the above-mentioned bacteria, there are many other bacterial stains and genera that have been shown to degrade a range of different plastic polymers with varying mechanisms and efficiencies like, some *Rhodococcus* species have been investigated for their ability to degrade PET. They produce enzymes like cutinases and esterases that are involved in plastic degradation (Zampolli et al., 2022; Qi et al., 2021). Certain Bacillus species have also demonstrated PET-degrading activity, these include, *Bacillus licheniformis* and *Bacillus subtilis*. These bacteria produce enzymes that can break down PET, although their efficiency and specificity may vary (Benavides et al., 2022; Qi et al., 2021). Others include *Nocardia* spp. and *Comamonas acidovorans*. Furthermore, studies and research are being carried out to isolate and discover novel bacterial species which can degrade plastic polymers and hold significant potential for future commercialization.

3. Fungi to the Rescue

3.1 *Aspergillus* spp.

Aspergillus is a fungal genus consisting of various species with high genetic diversity. The ideal growth conditions for *Aspergillus* species exhibit variability based on the specific species. For instance, *Aspergillus flavus* thrives in arid and warm environments. Its optimal growth temperature is 24-37℃, yet it readily proliferates within the temperature range of 25-42℃. Moreover, this fungus can flourish at temperatures spanning 12-48℃ (Passamani et al., 2014).

Aspergillus species rank prominently among naturally occurring microorganisms capable of PET degradation in the wild. Within soil microorganisms, *Aspergillus* spp. have demonstrated their inherent ability to biodegrade PET, showcasing their potential for plastic breakdown. Both fungi and bacteria with PET-degrading capabilities possess specific enzymes like PETase, which are hydrolases specialized in breaking ester bonds within the plastic. The process of PET biodegradation, albeit gradual, prompts the utilization of preliminary treatments to enhance its overall efficiency. Fourier-Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) stand as the foremost techniques utilized for the investigation of PET degradation (Benavides et al., 2022).

Several notable species of *Aspergillus* which include: *A. nidulans, A. flavus, A. glaucus, A. oryzae* and *A. nomius*; exhibit remarkable proficiency in breaking down plastics. These fungi use various enzymes such as cutinase, lipase, proteases, and lignocellulolytic enzymes, along with the effect of some pro-oxidant ions, to achieve effective plastic degradation. The enzymatic oxidation or hydrolysis adds functional groups, increasing polymer hydrophilicity and consequently converting high molecular weight polymers into lower molecular weight compounds. This intricate process contributes to plastic degradation within a relatively short span, often spanning just a few days (Srikanth et al., 2022; Verma and Gupta, 2019).

The future of these species in PET degradation holds promising potential. Ongoing research aims to enhance their PET-degrading capabilities through enzyme optimization and genetic engineering. Harnessing *Aspergillus* enzymes for efficient PET breakdown could contribute to sustainable plastic waste management and inspire innovative eco-friendly solutions. Commercial applications may emerge, addressing environmental

concerns and advancing the field of biodegradation technology.

3.2 *Pleurotus* spp.

Pleurotus belongs to the basidiomycete category and is classified under the *Pleurotus* genus. Its mushroom fruiting structures exhibit unique forms such as shell, fan, or spatula, displaying varying colours like white, cream, grey, yellow, pink, or light brown, influenced by the specific species (Maurya et al., 2020 (b)). The ideal growth conditions for *Pleurotus* spp. vary by strain, yet certain favourable factors have been identified. A research study revealed that optimal conditions were established by assessing growth and primordium development across varying parameters: osmotic potential (-0.5 MPa to -5.0 MPa), temperature (5 to 40°C), and pH (2 to 12) (Gorai and Sharma, 2018).

*Pleurotu*s spp. is a type of fungi that is efficient at breaking down plastics like PET. In a research conducted, scientists studied *Pleurotus ostreatus* and *Pleurotus pulmonarius* to see if they could break down PET plastic in soil and rice straws. They mixed plastic with the substrates and fungi, then waited for 2 months. They used a special tool FTIR to check the plastic and saw changes after 30 and 60 days, showing the plastic was breaking down. They also found some new substances that formed during the breakdown and that these fungi can break down plastic by cutting its chains into smaller pieces (Odigbo et al., 2023; Srikant et al., 2022).

The enzymes secreted by *Pleurotus* spp. can cleave the ester bonds in PET, producing BHET, MHET, TPA, and EG. BHET and MHET are intermediate degradation products, with BHET being further hydrolyzed by the same enzymes. A study revealed that the enzymes secreted by the fungi during biodegradation cause an increase in the number of carboxyl-terminated molecules, which leads to the change in colour of PET flakes. Fungi express biodegradation enzymes like cutinase, lipase, esterase, and hemicellulase more effectively than bacteria (Qi et al., 2021; Benavides et al., 2022).

Pleurotus spp. holds promise for PET degradation due to its diverse strains adapting to optimal growth conditions. Continued research into enzymatic efficiency, scale-up, and industrial applications may propel *Pleurotus* spp. towards a sustainable future in PET plastic waste management.

3.3 *Penicillium* spp.

Penicillium is a group of saprophytic fungi, typically forming colonies, often with rapid growth, displaying diverse textures and colours. They grow best in a pH range of 3 to 7 and at room temperature (28°C) (Moss, 1987).

Moyses et al. in their study stated that filamentous fungi like *Penicillium restrictum* and *P. simplicissimum* display significant potential for PET degradation, with the latter demonstrating enhanced lipase production and biodegradation when induced with BHET. This induction led to a near two-fold increase in lipase output and a notable 3.09% mass reduction in PC-PET fragments after 28 days. Moreover, the organism-level mechanisms exhibited during microbial biodegradation outperformed enzymatic depolymerization, emphasizing the potential of *P. simplicissimum* as an efficient PC-PET biodegrader for feedstock recycling, holding promise for sustainable PET waste management and monomer recovery (Moyses et al., 2021).

In the process of biodegradation, these fungi exhibit effective colonization of PET surfaces, triggering microstructural alterations like hole formation, surface corrosion, and crystal growth (Sepperumal et al., 2013). *Penicillium* spp. exhibits the potential to modify PET surfaces through hydrolytic enzymes, including cutinases, lipases, proteases and lignocellulolytic enzymes. As a consequence of the enzyme's oxidation or hydrolysis, the large-molecular-weight polymer is transformed into a lower molecular weight polymer, generating functional groups that promote polymer hydrophilicity. Within a few days, this causes polymers to degrade (Srikanth et al., 2022).

A study by Liebminger et al. highlights a novel 'polyesterase' from *P. citrinum* that can effectively hydrolyze PET, offering possibilities for enhancing PET fabric hydrophilization. Screening for PET degradation involved incubating environmental samples with a PET model substrate, leading to the identification of *P. citrinum* as a potent degrader. The ability of the fungus to break down cutin and PET components demonstrates its potential in PET degradation. These fungi attack plastics by releasing hydrolases that cleave ester bonds, such as lipase, which are potent degrading enzymes (Benavides et al., 2022). Understanding these mechanisms could lead to improved PET recycling strategies, benefiting from the fungus's unique enzymatic capabilities (Liebminger et al., 2007).

3.4 Other Fungal spp.

Above mentioned are just a few species of PET degrading fungi, however, since fungal biodegradation presents a sustainable approach to reducing plastic pollution and advancing circular economy initiatives, studies are being conducted for better insight. Other fungal species which are known to degrade plastic polymers include *Trichoderma spp., Rhodococcus spp., Cladosporium cladosporioides, Bjerkanderaadusta, Phanerochaetechrysosporium*, and *Agaricus* spp. showing particular promise. Further exploration and innovation are likely to drive the integration of fungal-based PET degradation into broader waste management strategies, contributing to a more environmentally conscious and sustainable future (Srikant et al., 2022; Kim et al., 2022 (b)).

4. Algae to the Rescue

4.1 *Spirulina* spp.

Spirulina is an autotrophic, spiral-shaped microalgae with a blue-green hue. It is an alkaliphile with an optimum pH of 8.5 or higher and thrives at temperatures around 30℃ (AlFadhly et al., 2022). The degradation of PET microplastics by *Spirulina* sp. was reported to be 48.61% in the only study that used this combination (Benavides et al., 2022). Hadiyanto et al. reported that PET and salt affected the growth rate and nutrient removal rates of *Spirulina* spp. in culture, reducing them by 0.174 on the first day. However, the salinity sy*stem on medium-added PET indicated that Spirulina* spp. had some effects on PET, where PET might be degraded by the algae in the water with a salinity of 7 ppt (Hadiyanto et al., 2022). Not enough research has been carried out to demonstrate the PET degrading potential of *Spirulina*, therefore, its potential to be used as a plastic degrader remains an interest of further research.

5. PET degradation through wax worms

Apart from microbes, several insects have the capacity to breakdown strong polymers. The larvae of the lepidopteran *Plodia interpunctella* and *Galleria mellonella*, and of the coleopterans *Tenebrio molitor* and *Zophobas antrum* can hydrolyse PE (Yang et al., 2015).

Exploring insect gut microbiota revealed two strains, *Enterobacter asburiae* YT1 and *Bacillus* spp. YP1, capable of forming biofilms on PE surfaces. This caused sustained deterioration, reducing hydrophobicity and forming pits over 28 days. The larval gut's role in plastic degradation is debated, with speculation about potential internal mechanisms aiding in this process (Bertocchini & Arias, 2023) **(Figure 2)**.

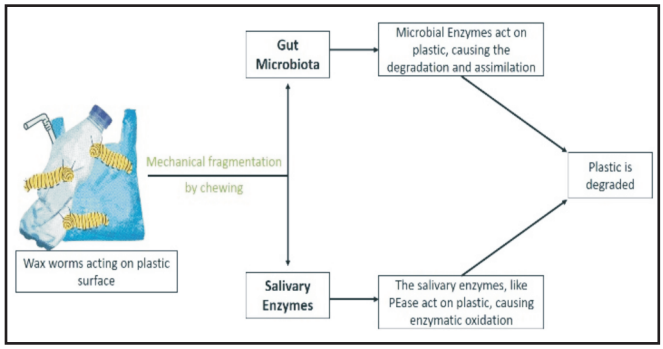

Figure 2: *The two types of biodegradative pathways carried out by wax worms for plastic biodegradation.*

Wax worms efficiently degrade PE using saliva, quickly oxidising the polymer and producing small oxidised molecules. Their saliva contains PEases, phenol oxidase enzymes that counteract plant phenolic compounds. Research findings suggest that the enzyme-rich buccal juice is protein-dense (10-15 nm), excluding other structures. Proteomic analysis identified over 200 proteins, including enzymatic, transport, and structural proteins. Size exclusion chromatography yielded a major fraction with a strong 75 kDa protein band, known in arthropods for transport and storage functions (Bertocchini & Arias, 2023; Sanluis-Verdes et al., 2022).

6. Conclusion and Future Prospects

The future prospects of microbial degradation of PET plastic are highly promising, with several avenues of research and development, showcasing potential breakthroughs. Ongoing studies focus on enhancing the efficiency of PET-degrading enzymes such as PETase and MHETase through advanced enzymatic engineering, aiming to improve their activity, stability, and specificity. Another avenue involves investigating microbial consortia that collaborate to synergistically degrade PET, potentially expediting the breakdown of PET waste. Synthetic biology offers a unique approach, allowing for the genetic engineering of specialized microorganisms with optimized PET degradation pathways, leading to more targeted and efficient degradation processes. The development of bioreactor technology is also underway, with the goal of providing optimal conditions for PET-degrading microorganisms, thereby enhancing degradation rates and scalability.

Metagenomics presents an innovative strategy by exploring diverse environments for naturally occurring PET-degrading microorganisms. This approach, utilizing metagenomic techniques, has the potential to uncover new and efficient candidates for plastic degradation. Beyond laboratory settings, the integration of microbial PET degradation into real-world applications is explored, including wastewater treatment systems and remediation efforts for plastic-contaminated environments, providing sustainable waste management solutions.

The potential impact extends to bioplastic production, where harnessing microbial degradation pathways may facilitate the synthesis of biodegradable plastics from PET waste or renewable sources, contributing to the reduction of plastic pollution. This aligns with the broader concept of a circular economy, where microbial PET degradation becomes an integral part of models aiming to minimize waste, promote resource efficiency, and reduce environmental impact by closing the loop on material use. As research advances, the collaboration between academia, industry, and startups holds the promise of translating these innovations into commercially viable PET degradation technologies.

In conclusion, the future of microbial PET degradation relies on a comprehensive strategy that integrates enzyme engineering, bioreactor technologies, microbial consortia, and metagenomics. This holistic approach contributes to a more sustainable and eco-friendlier paradigm in plastic waste management. However, the utilization of microorganisms for PET degradation encounters

challenges in optimizing enzymatic efficiency. Enhancing PETase and MHETase is intricate due to complexities in stability and specificity. Developing synergistic microbial consortia requires a nuanced understanding of inter-microbial dynamics. Synthetic biology grapples with dilemmas in balancing genetic modifications with ecological concerns, while bioreactor technology demands precise environmental control. Metagenomic exploration faces difficulties in isolating and characterizing microorganisms from complex communities. Overcoming these challenges is pivotal for unlocking the full potential of microorganisms in PET degradation, thus propelling the advancement of sustainable solutions for plastic waste management.

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