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Biological Degradation of Polyethyleneterephthalate (Pet) By Selected Microorganisms And Microbial Enzyme

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Abstract: *Plastic-based pollution is increasingly acknowledged as one of the major environmental dangers on a global scale. Polyethylene Terephthalate (PET) is a key component of plastics; its overabundance as garbage is a significant environmental concern. The majority of the time, PET contamination is controlled via mechanical, thermal, and chemical-based treatments. However, these techniques either cost a lot of money or produce extra pollutants. As a result, an economical and environmentally responsible solution is required for the proper handling of waste PET-based plastics. In light of this, recycling or microorganism-based degradation is one of the key strategies for reducing PET pollution. For the treatment of PET wastes, various bacterial isolates, fungal species, and microbial enzymes have been investigated. These bacteria and enzymes operate on PET to stop it from breaking down into monomeric units, which then causes weight loss. A brief overview of the application of certain bacteria, fungi, microalgae, and microbial enzymes for the management of PET wastes is provided in the current review.*

Keywords: Plastic pollution, polyethylene terephthalate, degradation, microorganism and microbial enzymes

1. INTRODUCTION

Plastic waste is widely present in the environment as a result of improper disposal practices and indiscriminate use of plastics and associated items. Plastic has become so indispensable that it is now considered one of the indivisible commodities (Koshti et al., 2018). Due to the strong demand for plastic since the beginning of the 21st century, production has expanded significantly. As a result, plastic trash generation has also tripled in these two decades (Beat Plastic Pollution, 2020). 90% of the plastic waste created today, which numbers around 0.3 billion, ends up in the ocean (Schmidt *et al*., 2017). Since the 1950s, around 8,300 million plastic wastes have been generated, and by 2050, it is expected to reach double-digit billions, if plastic waste is generated at the same pace (Geyer *et al.,* 2017).

Plastics are a top choice for many industrial applications because of their resistance to ionizing radiation, oxidation, and organic solvents. 33 percent of the total plastic production is used for packaging (Rhodes, 2018). PET-based polymers stand out among the numerous types of plastic because they are frequently employed in the packaging industry due to their toughness and thermostability. PET is a transparent, colorless, semicrystalline resin with outstanding wear and tear resistance, tensile strength, and transparency qualities (Koshti et al., 2018). Because of these qualities, PET is widely employed in the packaging industry. It is widely utilized in plastic films, food jars, and soft drink bottles.

erephthalic acid (TPA) and ethylene glycol (EG) are polycondensed to create polyethylene terephthalate, or dimethyl terephthalate and EG are transesterified to create a polymer of semiaromatic polyesters (Hiraga *et al*., 2019). Ester connections connect the PET's TPA and EG monomeric units. Its hydrophobic nature and chemical inertness produce a nearly impermeable surface (de Castro *et al*., 2017). PET is known to have a 240–250 oC melting point (Tm) and strong hydrolytic stability (Mohsin et al., 2017). Low crystalline PET (lcPET), which has a crystallinity of up to 7%, and high crystalline PET (hcPET), which has a crystallinity of between 30 and 35 percent, are two different types of PET (Furukawa *et al*., 2019). The amount of CrI shows how mobile the ester connections are in PET (Zekriardehani et al., 2017). More stiffness in the links is indicated by high CrI. PET has a glass transition temperature (Tg) of roughly 70 to 80 ^{o}C. The glass transition temperature, or Tg, is the temperature at which the polymer becomes more mobile and more accessible to ester bonds between monomeric units.

Out of the 269 million tons of total plastic production, 18.8 million tons were produced in 2015 due to the increased demand for PET-based plastics, particularly in the packaging industry (Taniguchi *et al*., 2019). Only 28.4% of the entire amount of PET produced gets recycled into fiber, sheets, films, and bottles; the remainder is thrown away into the environment (Taniguchi *et al*., 2019). This abandoned PET subsequently enters the environment and poses a risk to numerous life types. PET is typically not biodegradable and has a high crystallinity, making it particularly difficult to disintegrate. In light of this, the majority of PET-based plastic waste is either burned or disposed of in landfills (Geyer *et al*., 2017).

Aquatic animals who consume small plastic particles floating on water and stray animals who consume plastic materials cause numerous physiological changes in these creatures (Bhattacharya and Khare, 2020). This consumption can occasionally cause blockages in the digestive tract and obstructions in the respiratory passages, both of which contribute to the eventual demise of a particular animal species (Koshti *et al*., 2018). Additionally, the toxic components produced during the partial decomposition of plastic wastes contribute to soil pollution and have an adverse effect on a variety of life forms.

Due to their hydrophobic character, PET-based materials serve as adsorption sites for a variety of contaminants, including persistent organic pollutants and heavy metals prevalent in aquatic and terrestrial systems (Bhattacharya and Khare, 2020). Due to their potential to become biomagnified via food chain transmission, these attached poisons pose a concern to consumers at the top trophic levels (Koshti *et al*., 2018).

Landfilling and incineration are now the two most widely used techniques for disposing of plastic and PET in underdeveloped nations. Landfilling is impractical because to space constraints and rising costs, and incineration emits poisonous gases including a variety of toxicants and fly ash that must be disposed of further (Saleem *et al*., 2018). Recycling is thought to be one of the best ways to handle plastic/PET waste, though. Comparing the manufacture of recycled PET with that of virgin PET made from petrochemicals, the carbon footprint of recycling is reduced (Quartinello *et al*., 2017).

Following consumption, PET waste is recycled to create new products by recovering PET monomers in several nations (much of Europe and Japan) (TPA and EG). PET trash is often managed using a variety of recycling techniques, including thermal (used as fuels), material/mechanical (melted and reused once), and chemical/catalytic (degraded to monomers and utilized for resynthesis) (Kawai *et al*., 2019).

The biological breakdown of PET is regarded as a green method since it minimizes PET waste, adopts an environmentally favorable strategy, and is simple to use (Taniguchi *et al*., 2019). Because of its benefits to the environment and economy, biodegradation is chosen (Farzi *et al*., 2017). Microbes produce water-soluble intermediates and extracellular enzymes to depolymerize PET. Microorganisms use these intermediates for further metabolism and degradation (Gong *et al*., 2018). The ester group increases PET's resistance to biodegradation. The extracellular enzymes cutinase, lipase, PETase, protease, and esterase are among the PET-degrading microorganisms that have been identified thus far (Janczak *et al*., 2018; Dbrowska *et al*., 2021). This review is concerned with the biological breakdown of polyethylene terephthalate by certain bacteria, fungi, microalgae, and microbial enzymes.

2. Selected Microorganisms Involved In Degradation of Polyethleneterephthalate

2.1 Escherichia coli

Due to its clear genetic background, straightforward growing requirements, and advantages in high density cultivation, E. coli is a crucial model bacterium for the creation of recombinant proteins. More and more enzymes have successfully expressed themselves in a heterologous manner in E. coli in recent years as a result of the ongoing identification of PET hydrolases (Tournier *et al.*, 2020; Palm *et al*., 2019; Samak *et al*., 2020) summarized the PET hydrolases heterologously expressed in E. coli, which is useful for delving deeper into the crystal structures of these enzymes and examining the PET degradation mechanism.

Recent research has demonstrated the potential of modified E. coli as a whole-cell biocatalyst for PET biodegradation. The section of heterologous PET hydrolases is frequently improved by choosing the best signal peptide. In a study, the effects of Sec-dependent and SRP-dependent signal peptides from E. coli on secreting PETase were examined. SPLamB and PETase were successfully fused to yield 6.2 mg/L of PETase (Seo *et al*., 2019).

By altering the signal peptide, additional studies increased the expression level and enzymatic activity. In order to express heterologous PETase in E. coli, researchers successfully exploited an evolved signal peptide PelB (G58A) acquired from random mutation. This allowed for up to 1.7-fold greater PETase secretion (Shi *et al*., 2021). In order to mediate the excretion of PETase, an enhancer of signal peptides B1 (MERACVAV) was explored. Ultimately, the excretion efficiency of PETase mediated by B1PelB showed a 62-fold increase over that of PelB (Cui *et al*., 2021).

2.2 Bacillus subtilis

Gram-positive Compared to E. coli, which typically forms an inclusion body, B. subtilis is thought to be a suitable microbial chassis for secreting heterologous proteins because of its high secretion capacity, quick growth, and lack of an outer membrane (Van Dijl and Hecker, 2013). B. subtilis is regarded as a promising microbial chassis for biodegradation since it has a strong tolerance to hostile conditions and has been used to release proteins that can digest a variety of contaminants (Haung *et al*., 2015).

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Another two PET hydrolases (BhrPETase and LCC) were also expressed in B. subtilis, and the expression titer of BhrPETase and LCC reached 0.66 g/L and 0.89 g/L in an engineered chaperoneoverexpression of B. subtilis, respectively (Xi et al., 2021). Additionally, the combinations of signal peptides and promoters were optimized to promote the expression of PETase in B. subtilis WB600, and the combination of the signal peptide SPamy and the weak promoter P43 was proved to be best (Wang et al., 2020).

2.3 Thermophilic Bacteria

The majority of the hydrolases that can break down PET, such as lipases, cutinases, and esterases, have higher enzymatic activity at higher temperatures, whereas the majority of model microorganisms that may manufacture heterologous PET hydrolases typically prefer a growth temperature of 30 to 40ºC. Some PET hydrolases that are active exclusively at high temperatures are incompatible with whole-cell biocatalyst (Guyot *et al.,* 2014). Therefore, a thermophilic expression system is required to increase the biodegradation efficiency of PET.
LCC has been successfully produced using a C. thermocellum that was developed. After 14 days, our designed whole-cell biocatalyst achieved a high level of LCC expression and turned more than 60% of a commercial PET film into soluble monomers at 60°C (Yan *et al*., 2021).
This thermophilic whole-cell degradation system is a potential approach to degrade PET using other high temperature hydrolases since it has the benefit of simultaneous enzyme synthesis and PET degradation as opposed to merely employing free enzymes (Sooch et al., 2016). Alkali-tolerant whole-cell catalytic systems as well as the thermophilic whole-cell degradation system have both been reported (Gong *et al*., 2018).

2.4. Fungi

In addition to bacteria, some yeast, such as Pichia pastoris and Yarrowia lipolytica, may also be employed in the biodegradation of PET. With excellent secretion expression and scalable fermentation capabilities, P. pastoris has emerged as a popular strain for protein production in industrial applications. When PETase (H344S/F348I) and BurPL (H344S/F348I) were expressed in P. pastoris and E. coli, researchers found that P. pastoris's protein half-life protection mechanism caused both enzymes to have higher activity than those expressed in E. coli (Xu *et al*., 2020).
By putting PETase on the surface of P. pastoris, a whole-cell biocatalyst was created, and when compared to isolated PETase, its enzymatic activity rose 36-fold toward a highly crystalline PET. Furthermore, this whole-cell biocatalyst has a seven-fold reusability limit with no discernible activity loss, which is beneficial for creating new whole-cell biocatalysts for PET biodegradation (Chen *et al*., 2020). Researchers examined the impact of glycosylation on the LCC expressed in P. pastoris in light of the organism's capacity for N-linked glycosylation and discovered that the LCC's kinetic stability and activity were both increased (Shirk *et al.*, 2018).
Additionally, Y. lipolytica is an excellent microbial platform for bioremediation (Madzak, 2015). In order to demonstrate that Y. lipolytica is a potential microbial chassis for PET biodegradation, researcher's isolated Y. lipolytica IMUFRJ 50682 with the capacity to convert PET into MHET and confirmed that the PET monomers may act as inducers in the process of lipase production (Da Costa *et al*., 2020). Other research demonstrated that the modified strain could hydrolyze BHET and PET powder into the monomers by expressing PETase in Y. lipolytica Po1f with a signal peptide from lipase (Liu *et al*., 2021)

2.5. Marine Microalgae

Currently, the natural and engineered microbial chassis that can make PET hydrolases typically find it challenging to adapt to the complexity of the marine environment and generate a lot of PET waste. Recently, several marine microalgae have been utilized as the foundation for the breakdown of PET (Barone *et al*., 2020). The recombinant PETase was able to efficiently degrade various substrates, including PET films, poly (ethylene terephthalateco- 1,4-cylclohexylenedimethylene terephthalate) (PETG) film, and shredded PET, at 30ºC or even at mesophilic temperatures (21ºC), according to research on a photosynthetic microalga called Phaeodactylum tricornutum.
Additionally, Chlamydomonas reinhardtii, the green algae, was also successfully engineered to produce PETase with degrading activity, and the chemical and morphological changes appeared on the PET films after 4 weeks of culture (Kim *et al*., 2020). As environmentally friendly chassis for the biodegradation of PET waste in a saltwater-based environment, marine microalgae have the potential for future biotechnological applications in the degradation of PET polluted seawater (Moog *et al*., 2019).

3. Microbial Consortia in PET Biodegradation

Synthetic biology now prioritizes the study of artificial microbial consortia that mimic natural microbial consortia to carry out challenging biological activities (Qi *et al*., 2021; Ding *et al*., 2016). In particular, for the bioconversion of pollutants, it is crucial to investigate the potential and reprogram the functionality of microbial consortium members (Dangi et al., 2021). Utilizing artificial microbial consortia, previous investigations have demonstrated the potential for biodegradation and bioconversion (Skariyachan *et al*., 2021). Artificial microbial consortia have been utilized to increase the desulfurization of petroleum sulfides and degrade hydrocarbons (Ibrar and Zhang, 2020), organophosphorus insecticides (Sun et al., 2020), polyaromatic hydrocarbon pollutants, and aryl organophosphate flame retardants (aryl-OPFRs) (Martinez *et al*., 2016).

Additionally, some artificial microbial consortia for the degradation of plastic waste have been developed, including those for the degradation of polyurethane (PU) (Utomo *et al*., 2020), polyethylene (PE) (Syranidou *et al*., 2017), polypropylene (PP) (Aravinthan *et al*., 2018), and polyvinyl chloride (PVC) (Giacomucci *et al.,* 2020). These findings demonstrate the utility of synthetic microbial consortiums for PET biodegradation.

he use of artificial microbial consortia for PET biodegradation has a number of advantages over pure culture, including the following: (i) simultaneous PET biodegradation and bioconversion by multiple microorganisms; (ii) the ease with which artificial microbial consortia can be built; and (iii) the reduction of inhibitory effects on degradation products (Ballerstedt et al., 2021). The use of artificial microbial consortia in the biodegradation and bioconversion of PET is therefore seen as a viable strategy to accomplish the circular economy of PET waste.

3.1. Natural Microbial Consortia in PET Biodegradation

The majority of microbial consortiums that can now break down PET are natural microbial consortiums. Three Pseudomonas species and two Bacillus species were part of a consortium that was discovered by researchers to be able to reduce the weight of granular PET. After being exposed to the consortium for six weeks, a 100 mg granule of PET weighed 3.15 mg less, suggesting that the strains may work together to degrade PET (Leon-Zayas *et al*., 2019). Next, scientists looked for lipase activity linked to PET biodegradation, and they demonstrated that the secreted enzymes obtained from the consortium could completely convert BHET into TPA and EG (Roberts *et al*., 2020).

A consortium from activated sludge, containing Bacillus cereus SEHD031MH and Agromyces mediolanus PNP3, was described in another investigation. The collaboration could employ PET microplastics (MPs) as the only carbon source and decompose 17% of PET MPs over the period of 168 days at 30 °C, according to the report (Torena *et al*., 2021). Oberbeckmann *et al*. (2016) also examined the effects of various seasons, geographical locations, seawater, and substrate material types on the microbial consortia that used single-use PET bottles at numerous sites in the North Sea.

Cutinases, lipases, and esterases are the majority of PET hydrolases that have been previously reported, and they can only partially degrade PET. A microbial consortium No. 46 that totally decomposed amorphous PET from a waste recycling station at room temperature was successfully identified by Yoshida et al.(2016). Then, from the No. 46 consortium, a bacterium called I. sakaiensis 201-F6 was discovered that could break down and absorb PET. It could produce PETase and MHETase to break down PET, opening up a new avenue for PET biodegradation at room temperature (Taniguchi *et al*., 2019).

Marine microbial consortia can colonize PET, form biofilms on its surface, and finally modify its chemical structure (Pinto *et al*., 2019). A study demonstrated for the first time that hydrocarbon degrading marine consortia enriched on tetradecane and diesel have the potential to degrade PET and cause major alterations to the surface structure and hydrophobicity of PET films (Denaro *et al*., 2020).

3.2. Artificial Microbial Consortia in PET Biodegradation

There are currently few investigations on the development of synthetic microbial consortiums for PET degradation. After 4 weeks, CAS6, a unique three-consortium isolated from an ocean bay, can cause PET films to lose their crisp morphology in comparison to controls. Exiguobacterium sp., Halomonas sp., and Ochrobactrum sp. were the three bacteria that were eventually isolated from CAS6 and created a stable artificial three-microbial consortium in a 1:1:1 ratio to effectively destroy PET films. The three-microbial consortium incubated PET films for two weeks, during which time they completely decomposed into minute fragments (Gao *et al*., 2021).

In order to break down PET, Pan *et al*. (2021) created and engineered Y. lipolytica to secrete PETase, as well as a modified Pseudomonas stutzerithe to turn TPA into PHB. Over the course of 54 hours, they created a microbial consortium using two engineered strains to convert BHET into PHB. This was the first time PET had been simultaneously hydrolyzed by enzyme and converted to TPA. PETase's poor hydrolyzing efficiency prevented PHB from being made directly from PET, but it did show that synthetic microbial consortia were capable of simultaneously degrading and recycling PET (Pan *et al*., 2021).

The weight loss of PET film reached 23.2% under ambient temperature when Qi *et al*. (2021) created a four-species microbial consortium made up of two metabolically modified B. subtilis, *Rhodococcus jostii*, and *P. putida* (Qi *et al.*, 2021). The artificial microbial consortia efficiently increased the degradation rate and removed the metabolic inhibition of TPA and EG (Qi et al., 2021).

4. Microbial Enzymes used for PET Hydrolysis

4.1 Cutinase

Cutinase (E.C. 3.1.1.74) is majorly produced by either saprophytic microorganism, which utilizes cutin as a carbon source or by phytopathogenic microorganisms for breaking the cutin barrier to enter into the host plants (Maurya et al., 2022). Cutinase is a serine esterase that has the catalytic triad consisting of Ser–His–Asp residues. It belongs to the/b hydrolase superfamily. The active site of cutinase can accommodate high-molecular-weight compounds such as cutin and other related synthetic compounds (Maurya et al., 2022).

Hydrolysis of synthetic polymers such as PET (Dimarogona *et al*., 2015), polycaprolactone (Adıgüzel and Tunçer, 2017), polystyrene (PS) (Ho et al., 2018), polyethylene furanoate (Weinberger et al., 2017), and polybutylene succinate (Hu et al., 2016) have also been reported using cutinase. Cutinase-mediated hydrolysis of polylactic acid is also demonstrated by several authors (Kitadokoro et al., 2019).
Cutinase possesses valuable properties particularly required for PET degradation, and thus, it has caught the eye of many researchers in recent years (Maurya et al., 2022). It is a well-studied substitute for harsh chemicals usually practiced during chemical-based hydrolysis/recycling of plastics (Tournier et al., 2020). Cutinases are also known to synthesize polyesters under non-aqueous media using polycondensation reaction with various diacids and alcohols. Similarly, Pellis et al. (2016) used cutinase 1 from Thermobifida cellulosilytica for polycondensation of dimethyl adipate with various polyols for the synthesis of high-molecular-weight polyesters.

4.2 Lipase
Lipase has also been used by several researchers for the hydrolysis of PET. Effective degradation of PET nanoparticles using lipase from Candida cylindracea and Pseudomonas sp. has been reported by Ma et al. (2012). Similarly, Wang et al. (2008) employ BHET/TPA-induced lipase from Aspergillus oryzae for hydrolysis of PET. Moreover, Carniel et al. (2017) and de Castro et al. (2017) used the combination of lipase from Candida Antarctica (C. antarctica lipase lB CALB) and HiC for efficient PET hydrolysis to TPA. Although HiC showed better performance with PET hydrolysis, the enzyme has limited competence to convert MHET (one of the intermediates of PET hydrolysis) into TPA. On the other hand, CALB can easily convert MHET into TPA but has lower efficiency toward initial PET hydrolysis when used singly (Maurya et al., 2022).
However, the combination of both enzymes synergistically improves the overall PET hydrolysis. However, complete studies on the effect of enzyme dosages, temperature, and pH are lacking. Lipase and cutinase have a common feature of surface hydrophobicity (de Castro et al., 2017). Unlike other lipases, lipase B has a superficial catalytic site; hence, in the absence of the hydrophobic interface, it is still accessible to the substrate (Stauch et al., 2015).

4.3 Esterase
Monomers of PET are linked by ester linkage, and these can be cleaved using esterase found in almost all living organisms (Koshti et al., 2018). Ribitsch et al. (2011) used Bacillus subtilis nitrobenzylesterase (BsEstB) and applied it to hydrolyze PET into TPA and MHET [mono(2-hydroxyethyl)] TPA. Kawai et al. (2014) made use of recombinant thermostabilized polyesterase from Saccharomonospora viridis AHK190 capable of hydrolyzing PET and the PET-hydrolyzing activity was observed to increase in presence of Ca ions. Recombinant esterase from Thermobifida halotolerans (Thh_Est) was reported by Ribitsch et al. (2012) to degrade PET into TA and MHET.

4.4 PETase
PETase (3.1.1.101) was discovered from the bacterium I. sakaiensis 201-F6 by Yoshida et al. (2016). PETase and cutinases share high sequence identity, indicating the existence of critical structural features responsible for substrate binding (Fecker et al., 2018; Kawai et al., 2019). Even small differences between these enzymes are crucial and define their specific activities (Chen et al., 2018). High-resolution crystal structure study of PETase highlights the active site, which seems to be wider than the other cutinases, and thus this could be a factor of the high specificity of the enzyme toward heavy substrate PET (Chen et al., 2018; Kawai et al., 2020). Overall, PET hydrolases (PET-hydrolyzing enzymes) are generally limited to cutinases; structurally, they are homologous to lipase, but lack a lid covering the active site (Kawai et al., 2019). This shallow open active site with hydrophobic amino acid residues aids in PET binding and hydrolysis (Kawai et al., 2020). The lid is present in the active site of lipase and is known for interfacial activation in lipases. Lipases are not much active in PET hydrolysis, but like esterases and cutinases, they are known for surface modification of PET fibers. Esterase activity is limited to short-chain acyl esters and thus is also not much reported to hydrolyze hydrophobic PET (Maurya et al., 2022). Comparative X-ray crystallography data of actinomycetes cutinases and PETase (from I. sakaiensis) showed the presence of a broader active site and extra disulfide bond in the latter (Kawai et al., 2020).
Also, the active form of cutinases is in the form of a Ca2C- bound state. There is no Ca2C-binding site in the case of PETase. Moreover, serine residue in the catalytic triad of actinomycetes cutinases is replaced with alanine in PETase (Kawai et al., 2020). However, compared to actinomycetes cutinases, PETase is heat liable and act only on lcPET. Considering this, presently researchers are trying to increase the thermostability of PETase and its catalytic efficiency using various protein engineering techniques (Kawai et al., 2020).
The textile or clothing industry is also one of the major producers of PET waste, as it uses polyester as a major raw material. However, the heterogeneous nature of textile waste creates a major hurdle in recycling, as it comprised different types of natural or synthetic plastic wastes. Chemical and mechanical recycling, though, is practiced, but segregation is the first and utmost important step in the recycling of textile wastes (Maurya et al., 2022). Biocatalytic recycling of textile waste though has potential, but there are limited reports on this aspect. As enzymes are highly specific and thus may target the suitable substrate (PET) in a heterogeneous kind of

waste, in this regard, sequential chemical treatment under neutral condition followed by enzymatic treatment for efficient hydrolysis of polyester composed textile waste is reported by Quartinello et al. (2017).

Chemical treatment under neutral condition resulted in the production of 85% TA and small oligomers (Quartinello et al., 2017). The oligomers were further hydrolyzed using enzymatic treatment utilizing HiC, yielding 97% of pure TA, available for further recycling. The mixture of PET hydrolases (as mentioned above) could also be used for the biocatalytic conversion of textile polymers into monomers for further recycling (Maurya et al., 2022). Moreover, compared to other cutinases, actinomycetes cutinases are known to have broad substrate specificity and thus could be used for hydrolysis of a range of polyesters fibers (Kawai et al., 2019).

5. Fungal Enzymes Involved in PET Biodegradation

In recent times, several studies have looked for enzymes involved in PET biodegradation. However, most studies have focused on bacterial enzymes (Gao et al., 2021; Taniguchi et al., 2019), with fungal enzymes being less investigated. The main fungal enzymes involved in PET biodegradation are hydrolytic enzymes acting on ester bonds (esterases; EC 3.1.1), such as cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3) and carboxylesterases (EC 3.1.1.1) (Carr et al., 2020).

5.1. Cutinases Involved in PET Biodegradation

Specific cutinases able to degrade PET were identified from Humicola insolens (HiC) (Taniguchi et al., 2019), Fusarium solani pisi (FsC) and Fusarium oxysporum (FoCut5a) ((Taniguchi et al., 2019). The most studied enzymes are the cutinases HiC and FsC. HiC has good thermostability with a temperature range from 30 to 85ºC, an optimum at 80ºC, and maximum initial activity from 70 to 80ºC. On the other hand, FsC has a lower temperature range of 30–60ºC with the best performance at 50ºC.

Ronkvist et al. (2009) tested the biodegradation capacity of HiC and FsC. They found that the hydrolysis rate constant k2 was 7-fold higher for HiC at 70ºC than FsC at 40ºC (0.62 µmol/cm2/h compared to 0.09 µmol/cm2/h). Moreover, the results showed a 97±3% weight loss when low-crystallinity PET was incubated with HiC for 96 h at 70 ºC, while there was only a 5% decrease after 96 h of incubation with FsC at 40ºC.

A few studies noted that the activity of cutinases was higher for an amorphous PET polymer compared to that of a highly crystalline substrate. Indeed, these enzymes are sensitive to chain distribution and length (Ping et al., 2017). An increase in the PET crystallinity rate from 7% to 35% caused a decrease in the initial enzymatic activities up to 25-fold for HiC and 6-fold for FsC. The enzymes' preference for amorphous regions of PET led to an increase in the biodegradation of these regions and an increase in the crystallinity rate of the biodegraded polymer. Moreover, a high presence of aromatic rings lowers the rate of hydrolysis. On the other hand, HiC preferably hydrolysed both internal (terephthalic acid-1,4-butanediol) and external (benzoic acid-1,4 butanediol) ester bonds, and more rapidly hydrolysed substrates with longer terminal alcohols but shorter chain length acids (Perz et al., 2016)

Another cutinase with potential in PET bioremediation is produced by Fusarium oxysporum, and is called FoCut5a (Dimarogona et al., 2015). It is highly homologous to F. solani pisi cutinase (FsC), but the hydrophobic residues Ala62 and Phe63 present in F. solani are replaced by Lys63 and Tyr64 polar amino acids in FoCut5a at the end of helix a2. Due to these and other small but significant differences, FoCut5a seems slightly more thermostable than FsC, underlining a possible important role in industrial applications (Dimarogona et al., 2015). The optimized parameters for PET hydrolysis are 40ºC, pH 8 and 1.92 mg FoCut5a per gram of fabric (Kanelli *et al*., 2015). FoCut5a efficacy was confirmed by superficial changes observable by Fourier-transform infrared spectroscopy (FTIR) ATR analysis, X-ray photoelectron spectroscopy (XPS), Scanning Electron Microscope (SEM), as well as through dyeability tests using reactive dyes(Kanelli *et al*., 2015).

5.2. Lipases Involved in PET Biodegradation

Lipases are another class of enzymes involved in PET biodegradation (Carr et al., 2020). The most studied that are involved in PET biodegradation are produced by Aspergillus oryzae CCUG 33812 and by the yeasts Candida antarctica (CALB) (De Castro et al., 2017) and Pichia pastoris (Gao et al., 2017). Aspergillus oryzae CCUG 33812 can produce a lipase able to catalyse PET hydrolysis using 0.1 g/L bis(2-hydroxyethyl) terephthalate (BHT) as an inducer. An increase in hydrophilicity and antistatic ability, as well as a 0.74% weight loss and a decrease in both the water contact angle and static half decay time, were observed after 24 h at 55ºC (Gao et al., 2017).

The lipase triacylglycerol hydrolase produced by the yeast Pichia pastoris was able to modify the surface morphology of polyester fibres at 60ºC and at pH 7.5–8. Moreover, 7 h treatment with the combination of 10 g/L P. pastoris lipase and 0.5 g/L non-ionic surfactant JFC (a fatty alcohol polyoxyethylene ether) at 60ºC and pH 7.5 changed the surface morphology of the PET fibres and increased the number of hydrophilic groups (Gao et al., 2017).

5.3. Polyesterases Involved in PET Biodegradation

Extracellular polyesterases involved in PET hydrolyzation are secreted by Beauveria brongniartii and Penicillium citrinum grown on a medium containing cutin with molecular weight 14.1 kDa, temperature optimum 36ºC and pH 8.2 (Temporiti et al., 2022). Polyesterase from B. brongniartii released TPA during treatment of PET, while P. citrinum enzymatic activity liberates only low amounts of TPA in favour of BHET and MHET (Temporiti et al., 2022).

5.4. Synergic Action of Cutinase HiC and Lipase CALB

An important PET depolymerization enzyme that can act synergically with HiC is lipase B from Candida antarctica (CALB) (Carniel *et al*., 2017). HiC and CALB present two different activity profiles at the final stage of PET depolymerization. Indeed, TPA was the predominant molecule after 24 h of CALB action, while HiC very quickly converted BHET into MHET, but then TPA formation was slow (De Castro et al., 2017).

Despite this, when the two enzymes are used alone, HiC is more efficient than CALB in degrading PET (Carr et al., 2020), while a synergic action between HiC and CALB led to a more intense MHET consumption and TPA formation (De Castro et al., 2017). Better results were obtained by de Castro et al. (2017) using HiC and CALB sequentially. This method led to an initial release of MHET (HiC action at 60ºC), which was rapidly converted to TPA after CALB addition (37ºC), resulting in a degradation 141-times higher than when using the two enzymes at the same temperature (De Castro et al., 2017)

6. CONCLUSION

This review summarized the current advances of PET biodegradation and bioconversion from the four aspects of engineered enzymes, chassis, pathways, and consortia, which provide a basis for the construction of artificial microbial consortia to convert PET into high value chemicals. Artificial microbial consortium is a promising strategy in realizing the circular economy of PET waste. On the one hand, the artificial microbial consortia are expected to effectively release the competitive inhibition of monomers in the PET biodegradation and improve the degradation efficiency. On the other hand, the artificial microbial consortia can couple the biodegradation of PET with the bioconversion of high value chemicals from monomers to realize circular economy and sustainability.

Owing to the recent advancements in synthetic biology and metabolic engineering, it has now become possible to rationally design and create artificial microbial consortia with a superior metabolic efficiency to degrade PET and convert it into high value chemicals in one step. Among various available methods known for recycling PET, enzymatic methods are considered an environmentally safer band efficient method for managing the PET wastes. Enzyme prominently cutinases are proved to be quite effective in PET hydrolysis.

Recycling such plastics has become a tedious problem. Additionally, enzymatic treatment of mixed wastes arising from the textile industry too is a problem, as this enzyme needs to have broad substrate specificity. If the search for a novel thermostable PET-hydrolyzable enzyme capable of hydrolyzing hcPET with broad substrate specificity is fulfilled, then this will ultimately help in the overall curbing of plastic pollution in a sustainable pattern.

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