Fungal Enzymes for Polyethylene Terephthalate (PET) Degradation

Subjects: Area Studies

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The ubiquitous persistence of plastic waste in diverse forms and different environmental matrices is one of the main challenges that modern societies are facing at present. The exponential utilization and recalcitrance of synthetic plastics, including polyethylene terephthalate (PET), results in their extensive accumulation, which is a significant threat to the ecosystem. The growing amount of plastic waste ending up in landfills and oceans is alarming due to its possible adverse effects on biota. Thus, there is an urgent need to mitigate plastic waste to tackle the environmental crisis of plastic pollution. With regards to PET, there is a plethora of literature on the transportation route, ingestion, environmental fate, amount, and the adverse ecological and human health effects. Several studies have described the deployment of various microbial enzymes with much focus on bacterial-enzyme mediated removal and remediation of PET. However, there is a lack of consolidated studies on the exploitation of fungal enzymes for PET degradation. Herein, an effort has been made to cover this literature gap by spotlighting the fungi and their unique enzymes, e.g., esterases, lipases, and cutinases. These fungal enzymes have emerged as candidates for the development of biocatalytic PET degradation processes. The first half of this review is focused on fungal biocatalysts involved in the degradation of PET. The latter half explains three main aspects: (1) catalytic mechanism of PET hydrolysis in the presence of cutinases as a model fungal enzyme, (2) limitations hindering enzymatic PET biodegradation, and (3) strategies for enhancement of enzymatic PET biodegradation.

Keywords: Enzymes ; polyethylene terephthalate ; PET

1. Synthetic Plastics—Categories and PET

Considering the structural backbone, synthetic plastics have been broadly categorized into two groups, i.e., (1) plastics with a C–C backbone and (2) plastics with a C–O backbone (**Figure 1**). The first category of plastics is non-hydrolysable, and examples include polypropylene (PP) and polyethylene (PE), among others. These plastics contribute to 77% of the global market share. Furthermore, the minimally reactive C–C bonds in the backbone of polyesters are considered a significant obstacle to the biodegradation process [1]. The plastic materials in the second category with a C–O backbone are hydrolysable, and examples include polyethylene terephthalate (PET) and polyurethane (PU) among others and hold around 18% of the global market share [2][3][4]. Collectively, the global plastic market was valued at around $568.9 billion in 2019, which increased to $579.7 billion in 2020, and is expected to grow at a compound annual growth rate (CAGR) of 3.4% from 2021 to 2028 [5]. According to one estimate, until 2020, about 300 million tons (Mt) of plastic wastes was being produced annually, which has now escalated to 400 Mt annually. Further to this, the annual production of plastics is expected to double by 2035 (approx. 800 Mt) and reach 1600 Mt by 2050 [6][7]. Unfortunately, around 76% of the overall plastic production is handled as waste. Of this, 9% is recycled, 12% is incinerated, and 79% is landfilled or released to the environment [2][7].

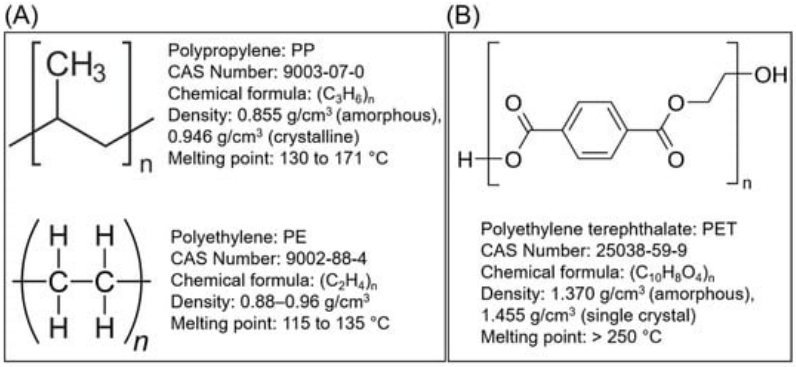

Figure 1. Structural and physicochemical characteristics; (**A**) category of non-hydrolysable plastics examples with a C–C backbone, and (**B**) category of hydrolysable plastics examples with a C–O backbone.

PET is the most common single-use plastic among various synthetic plastics and is considered a thermoplastic polymer resin of the polyester family. PET is a clear, strong, and lightweight plastic that is widely used for packaging (**Table 1**) [8][9][10][11][12]. According to the British Plastic Federation (BPF), over 70% of the soft drinks in the global market are being packaged in PET bottles [13].

Table 1. Polyethylene terephthalate (PET) packaging products based on end-user consumption. The global plastic consumption: 367 million tonnes, total PET packaging products consumption: 27 million tonnes in 2020 (7.4%). (Source: Data were extracted and calculated based on refs. [14][15][16][17][18]).

PET Packaging Products	Global Consumption in 2020 (Million Tonnes)
Water Bottles	**7.02**
Carbonated soft drink (CSD) bottles (e.g., Coca Cola, beers)	**7.02**
Other drinks (e.g., juices, milk)	**4.86**
Other bottles/containers in form of films and sheets	**3.78**
Food containers	**2.43**
Containers for non-food consumer products (e.g., cosmetics)	**1.62**

Antimony (Sb), a metalloid element, is used as a catalyst in the form of antimony trioxide (Sb_2O_3) or antimony triacetate in PET production. The WHO published a risk assessment for antimony in drinking water [19]. PET toxicity is typically associated with the leaching of Sb upon exposure to heat [20], thus it deserves careful consideration. Exposing PET to a thermal environment causes the leaching of antimony significantly, for example, into bottled water [21], possibly above US EPA maximum contamination levels [22]. As the presence of leached antimony in bottled water is a serious public health and safety concern, a detailed analysis of the published data on the presence, concentration, and leaching of PET is essential [23].

Zheng et al. [17] observed that plastic polymers with pure carbon backbones are particularly resistant to most degradation methods. While this is often true, it is aromatic polymers that tend to be resistant to degradation, despite the presence of bonds that are typically readily hydrolyzed [18]. PET is a classic example of such a polymer, i.e., although the ester bond that is part of PET can be easily broken, PET is resistant to degradation due to the presence of a high ratio of aromatic terephthalate units [24]. This necessitates their removal from the environment. For this purpose, numerous methods are used, such as photo-oxidation, thermal degradation, chemical degradation, and biodegradation of PET [25][26][27][28]. However, each of these methods has its own merits and limitations. PET, as a polyester, is more resistant to biodegradation due to its ester bond group compared to other polymers. Several new studies on PET biodegradation by microbes, i.e., bacteria and their enzyme systems have been reported [25][28][29][30]. A plethora of literature is available on the bacterial enzyme-assisted degradation of PET [28][29][30]. However, little is published about fungal enzyme-mediated PET degradation. So far, there is a lack of robust fungal enzyme-mediated processes capable of efficiently mitigating the PET plastic-based contamination effectively and efficiently from ecosystems. For this, there is an urgent need for the development of sensitive and reliable detection systems that can be applied to the land- and -water-based plastic contaminants. This will enable the robust identification of plastic value chain hot spots that pose the most significant environmental problems. Thus, herein, an effort has been made to cover this literature gap by spotlighting the fungal strains and their potential enzyme systems as potential robust catalytic tools to degrade PET.

2. Strategies to Enhance Enzyme-Based PET Biodegradation

2.1. Thermostable Enzymes

Hyperthermophile microbial strains with optimal activity and stability temperatures of >80 °C are important sources of high-temperature thermostable enzymes, so-called “thermo-zymes” (enzymes resistant to irreversible inactivation at high temperatures). Thermo-zymes are considered ideal candidates for catalytic processes that need to be operated at high temperatures. Several adaptive strategies can be followed to screen or synthesize enzymes giving them functionality in a

high-temperature environment. Engineering high-temperature enzymes for robust catalytic transformation reactions are well covered in the literature [31][32][33][34], thus it is not the focus of this review. Screening thermophiles and engineered high-temperature enzymes, several other methods, such as the exploitation of ionic liquids, or deployment of suitable modifiers such as Ca^{2+}, and various immobilization methods using robust support matrices have been adopted to increase the thermostability of PET hydrolases [35][36][37]. Thus, these thermophilic PET hydrolases could efficiently be used for PET biodegradation purposes. For example, the thermo-stability and catalytic activity of PET-degrading cutinase-like enzyme, Cut190, was boosted by high concentrations of Ca^{2+}, which is essential for efficient enzymatic hydrolysis of amorphous PET [37]. The Cut190, a member of the lipase family, encompasses an α/β hydrolase fold and a Ser-His-Asp catalytic triad, thus hydrolyzing the inner block of PET [37].

2.2. Use of Surfactants and Additives

The catalytic turnover of enzyme-based reactions can be facilitated/boosted by using various surfactant molecules or surface-active additives in the enzymatic hydrolysis. Surfactants stabilize the enzymes, thereby effectively preventing enzyme denaturation during hydrolysis, which is a significant limitation of enzymatic PET biodegradation. The supplemented surfactant molecules tend to bind with the enzymes and alter the secondary and tertiary structures or flexibility of the enzyme, thereby shielding the enzyme kinetic properties [38]. Furthermore, the integration of surfactant molecules in the reaction medium can additionally improve the dispersibility of PET particles and thus may increase the accessibility of the substrate to enzymes. As mentioned earlier, the limited accessibility to substrate-binding active sites of the enzymes causes low activity for PET hydrolysis. This phenomenon may be ascribed to the hydrophobic force that prevents the enzyme from directly accessing the substrate [39][40]. The accessibility of the substrate to enzymes is very important as the presence of hydrophobic forces between the PET surface and reaction substrate is one of the significant limitations of the entire PET biodegradation process [41].

One considerable way to tackle this issue of surface hydrophobic/hydrophilic balance and substrate accessibility is the interfacial activation employing surfactant [39]. Hence, increasing the surface hydrophilization of PET near the substrate-binding region should promote cutinase-PET interactions, in the presence of surfactants, which is essential for its enzyme-assisted biodegradation. The ends of polymer chains on the PET surface are expected to protrude or form a loop [42]. Surface hydrophilicity could be increased through the hydrolysis of these loops to carboxylic acid and hydroxyl residues. The overall PET degradation can be further escalated by PET surface modification that is performed by the available microbial culture or its PET hydrolytic enzymes. PET surface properties can be improved by introducing surface-active additives to the PET surface to increase its hydrophilicity. In this context, the PET biodegradation potential of fungal cutinase from *Fusarium solani* pisi was induced by using various surfactants, including sodium dodecyl sulfate or sodium lauryl sulfate (SDS), Triton X-100, Tween 20, and sodium taurodeoxycholate (TDOC) at different concentrations in the presence of 20 mM Tris–HCl buffer of pH 8 [43]. Furthermore, various substrates, i.e., p-nitrophenyl butyrate (pNPB), p-nitrophenyl palmitate (pNPP), tributyrin, and triolein were also used to initiate the reaction. The results showed 73.65% PET biodegradation by *Fusarium solani* pisi cutinase that released soluble hydrolysis products, i.e., BHET, MHET, TA, and 1,2-ethylene-mono-terephthalate-mono(2-hydroxyethyl terephthalate) (EMT). The released hydrolysis products were detected and confirmed by LC-MS analysis [43]. Likewise, the incorporation of additive molecules, such as hydrophobins which are cysteine-rich surface-active proteins produced by filamentous fungi, has also been used to increase enzymatic PET hydrolysis [44][45][46]. Espino-Rammer et al. [44] tested two hydrophobins (HFBs), HFB4 and HFB7 of *Trichoderma* spp., to enhance the rate of enzymatic hydrolysis of PET. Both HFB4 and HFB7 displayed a dosage-dependent stimulation effect on PET hydrolysis by cutinase from *Humicola insolens*. Moreover, the simultaneous addition of *Humicola insolens* cutinase (final concentration, 0.2 mg/mL) and HFB4 (concentrations from 0.05 to 50 mg/liter) to PET resulted in stimulation of the cutinase activity. This was observed by measuring the released soluble hydrolysis products, TA and MHET [44].

2.3. Enzyme Tailoring and Genetic Modification

The above-discussed shortcomings of enzymes can be overcome via enzyme tailoring and genetic modification practices. In addition, the tailored or genetically engineered enzyme-based catalysis offers multi-benefits, such as mild processing for complex and stable compounds, e.g., PET, and the capability to diminish reaction by-products or limit the generation of intermediate secondary products (that resist the enzymatic PET biodegradation) [47][32][34]. Moreover, the genomic modification settings/protocols that could enhance the PET biodegradation potential of enzymes, i.e., esterases, lipases, cutinases, and others need to be improved/modified. Several strategies, such as random mutagenesis and site-directed mutagenesis, genome editing, computational genomics and advanced computational modeling, structure-guided protein tailoring, and directed evolution are among the recent strategies that have been implemented to address the catalytic limitations of enzyme engineering (**Figure 2**) [47].

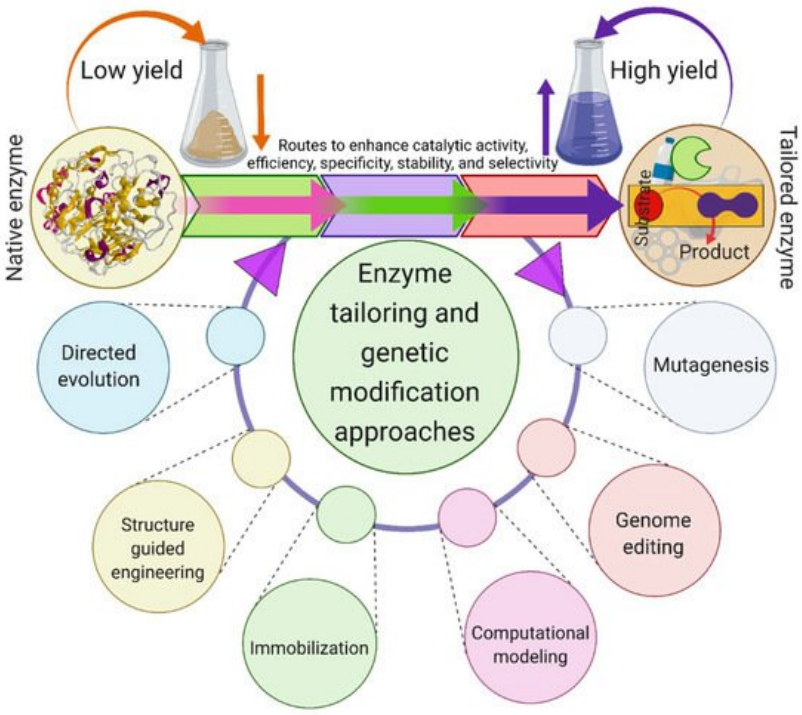

Figure 2. Protein engineering approaches to develop robust catalysts of interest with high catalytic activity, efficiency, specificity, stability, and selectivity, that could enhance PET biodegradation. Created with BioRender.com and extracted under premium membership.

Several PET-degrading enzymes, including cutinases, have been immobilized using different support matrices. Nevertheless, these engineered enzyme-based catalytic systems have been used in other applications rather than in PET hydrolysis. Hence, there are limited reports on PET hydrolysis using immobilized fungal hydrolases/cutinases. For example, Nikolaivits et al. [48] engineered cross-linked enzyme aggregates (CLEAs) of cutinase from *Fusarium oxysporum*. As discussed above, cutinases have been reported for PET biodegradation, hence, this CLEAs-cutinase from *Fusarium oxysporum* could also be used for PET hydrolysis. In another study, Su et al. [49] used Lewatit VP OC 1600 (a macro-porous divinylbenzene-crosslinked methacrylate esters resin) as solid support to immobilize three cutinases, i.e., cutinase from *Aspergillus oryzae*, cutinase from *Humicola insolens* (a thermophilic fungus), and cutinase from *Thielavia terrestris*. Essentially, the solubility and rigidity of PET polymers increase and decrease, respectively, in organic solvents, thereby allowing easy access of the engineered enzymes to ester bonds of PET for efficient hydrolysis. Hence, this immobilized HiC could also be used for the hydrolysis of PET [50].

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