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# PETase AND MHETase: A STEP TOWARDS PLASTIC DEGRADATION

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## ABSTRACT

Worldwide plastic waste disposal has taken the global attention due to annually elevated plastic output. Its ease of manufacture, strength, flexibility, and light weight make it the ideal for usage in both industrial settings and consumer goods. The most common polymers, Polyethylene terephthalate (PET), is produced at scale and used as a raw ingredient for plastic goods. Over time, the desirable versatility of a PET based plastic became a double-edged sword, as it is extremely difficult to recycle or decay. Globally, only microbial breakdown has been tested. Extracellular enzymes secreted by bacteria break down PET, the surface of the plastic is covered in biofilms due to the enzymes produced by various species and strain. Bacterial strain Ideonella sakaiensis 201-F6 was discovered in 2016 PETase and MHETase, enzymes have been isolated which are used as plastic degradation in present era. Many research has produced X-ray structures of both enzymes with various mutations and bound molecules, and have postulated what certain traits have contributed to the enhanced enzyme activity. It can be claimed that PETase and MHETase,provide distinct prospects for optimization as an effective biodegradation tool and address the problem posed by plastic wastes.

**Key words:** Polyethylene terephthalate (PET), polymers, double-edged sword, Ideonella sakaiensis, mutations, biodegradation tool

## INTRODUCTION

Environmental pollution has been currently a global attention caused by plastic waste. Between 1950 and 2015, an estimated 6,300 million metric tons (Mt) of plastic waste were produced. If present usage and management trends continued, $12\times10^9$ billion are anticipated to be dumped in landfills or contaminated wild areas and it is projected to reach 34 billion tons overall by 2050 (Geyer *et al*., 2017; Hachisuka *et al*., 2021). The versatility and low cost of plastics made them a necessity for human life during the 20th century (Worm *et al*., 2017). A new report from Plastics Europe estimated that Global plastic output increased by 5% annually 348 million metric tons in 2017, 359 million tons in 2018 this amount increased to 368 million tons in 2019. In 2016, 485 billion bottles were produced the estimated output rose to 583 billion bottles in 2021(Frias *et al*., 2021; Geyer *et al*., 2017). Overall 60% of the demand for plastic is accounted for by the construction and packaging sectors (Frias *et al*., 2021). Although they make our lives easier, P*Et al*so makes up 12% of the world's solid waste), and it seriously endangers our marine ecosystems (Bidegain and Paul-Pont., 2018). Plastic waste enters the ocean through a variety of channels, including waterways and beach and coastline littering with an estimated 4.8 to 12.7 Mt of plastic refuse coming from 192 coastal nations (Jambeck *et al*., 2015). Additionally, some plastic goods' ingredients have a negative effects on species found in soil, water, and on land, which raises questions about how they might affect human and animal health .The majority of plastics produced are single-use, improperly disposed-of products, which lead to accumulation in various ecosystems (Worm *et al*., 2017).

Hundreds of different materials with a broad range of properties make up the family of conventional plastics that are made from fossil fuels (Geyer *et al*., 2016). They are the perfect materials for use in a wide range of industrial and consumer products due to their flexibility, strength, lightweight, simple production, and low cost. Plastics are essential components in packaging, construction, transportation, medical equipment, etc. because of their versatility. By, for instance, offering superior barrier properties and thereby postponing food spoilage, plastics aid society in reducing food waste. Additionally, they enable energy savings in transportation, which lowers greenhouse gas (GHG) emissions. For instance, it is predicted that the use of plastic in automobile parts will have a fourfold greater positive effect on the environment than the use of other conventional materials. However, single-use plastics make up the vast bulk of the plastics produced. A little over 40% of all plastic manufactured is used for packaging. Thousands of seabirds, whales, marine turtles, and seals are killed each year by plastic debris in the world's oceans due to ingestion or entanglement. Previously seen as a benefit, plastic's high resilience and

persistence now cause an unchecked buildup of waste in every ecosystem on the world. Most plastics only break down into smaller fragments rather than disappearing entirely formed nanoplastics and microplastics (1 m–5 mm) (Waller *et al*., 2017). Additionally, it has been demonstrated that nanoplastics can enter fish's blood-brain barriers, disrupt behavior, and decrease marine zooplankton survival (Chan *et al*., 2017). According to a recent research, crop plants can efficiently absorb microplastic and transport it from the roots to the shoots. It comes out that the ubiquitous plastics have an impact on human health as well. In the lungs and fecal tissues, microplastics were discovered. Microplastics have been shown in in vitro studies to change membrane integrity, trigger an immune response, cause oxidative stress, cause cytotoxicity, and alter gene expression (Geyer *et al*., 2017). Microplastic contamination is another issue that is drawing increasing attention. Studies have shown that sea life is negatively impacted by environmentally relevant concentrations of microplastics, even though the precise amount is unknown. Additionally, it has been demonstrated that microplastics have an effect on the biological characteristics of soil, changing the way that soil functions. Therefore, rather than having a direct harmful impact, microplastics may cause a global change in terrestrial ecosystems (Li *et al*., 2020; Waller *et al*., 2017).

## POLYETHYLENE TEREPHTHALATE

One of the most prevalent polyester plastic manufactured worldwide is polyethylene terephthalate (PET) (Chen *et al*., 2018). PET is a synthetic polymer that is made on an enormous scale and used as a raw material for different plastic products including bottles, clothing, packaging carpeting, and single- use beverage bottles (Taniguchi *et al*., 2019). Over 50 million kilograms of PET are thought to be produced annually. Plastics manufactured in the largest quantities are PET, which accounts for 70% of total production (Puspitasari *et al*., 2021). Fibers made from PET are extremely durable, elasticity, strong, and chemical-resistant (Sini *et al*., 2021). Unfortunately, PET's inherent properties also make it difficult to degrade, resulting in an accumulation of the material in the environment (Chamas *et al*., 2020).

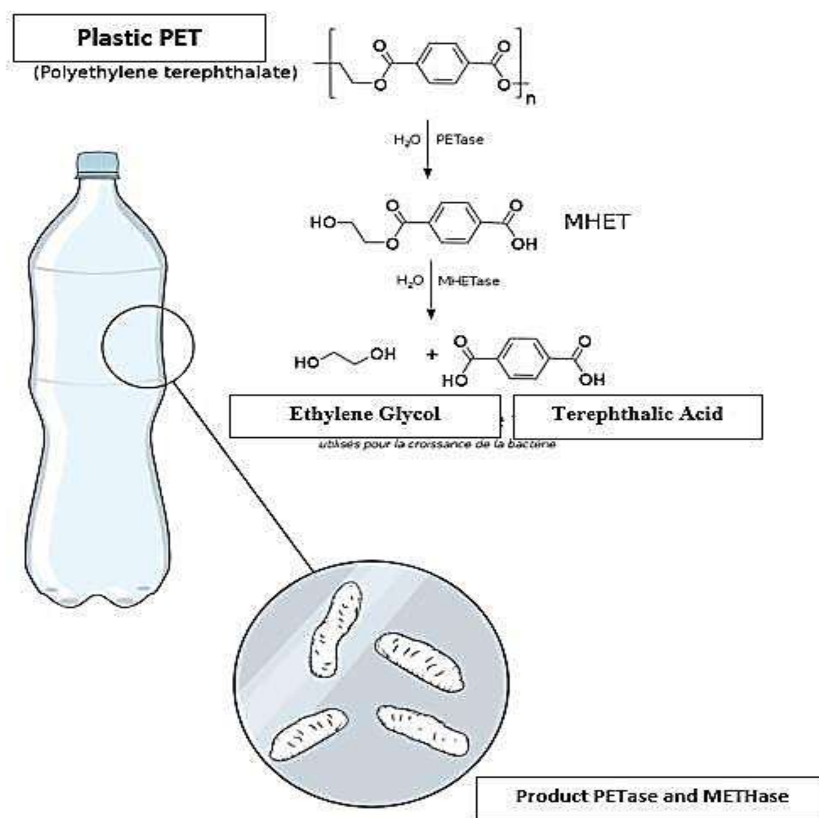


Fig.1. Polyethylene Terephthalate.

**Structural Characteristics**

Polyethylene terephthalate having three types of fibers acrylic, polyester and polyamide, polyester is the most prevalent type (Geyer *et al*., 2017). Further there are three types of polyesters are aliphatic, aliphatic-co-aromatic, and aromatic used by humans. PET, for instance, is a polyester that is semi aromatic (Austin *et al*., 2018). The commercial production of PET is the result of using dimethyl terephthalate (DMT) or terephthalic acid to polycondensate ethylene glycol. Since more than 20 years ago, researchers have been studying the enzymatic recycling of PET ( Weiland, 2020). Despite their relatively low crystallinity and glass transition temperatures, enzymatic access to ester linkages is relatively more direct. It's important to note that polyhydroxyalkanoates, polybutylene succinate, and polylactic acid, are among the aliphatic polyesters that can be manufactured from renewable resources (Austin *et al*., 2018). As a polymer made from petroleum, PET is semi-crystalline and exhibits both amorphous and crystalline characteristics. The crystalline regions are created when the longitudinal order of the polymer chains is maintained, which gives the material strength. In contrast, the amorphous polymer regions give the material flexibility because there is no longitudinal order. There are fewer enzymes available that can break C-C bonds in PET, along with its crystalline nature, making its biodegradation difficult. Although the ester connections in the amorphous regions are cleavable, as the crystallinity rises, access to the ester bond becomes more constrained (Taniguchi *et al*., 2019).

## HARMFUL EFFECT OF PLASTIC DISPOSAL

In the natural environment, PET is resistant to degradation due to its structural components, which restrict the movement of the polymer chain and increase surface hydrophobicity. PET can be recycled by manually shredding it into flakes and then reprocessing; however, this procedure heats the polymer, causing it to lose its mechanical qualities and making it impossible to reconstitute the original product. PET recycling has been described mechanically and chemically however these methods are expensive. Impact of these methods are negatively on the environment, so the development of more effective and environmentally friendly alternatives is urgently needed. Primary mechanical recycling of reclaimed PET eventually results in a loss of material properties and intrinsic value. An industrial setting using chemical process involving the cleavage of ester bonds is capable of depolymerizing PET into its constituents under harsh conditions, for example by using sulfuric acid at 150°C, or by using hazardous chemical catalysts (e.g., methyltrioctylammonium bromide) under alkaline conditions. Chemical recycling, on the other hand, includes chemically breaking down spent PET into monomers or partially depolymerized oligomers that can then be reassembled into polymers with similar characteristics. Even though this technique is a common closed-loop recycling one, it is criticized for producing secondary environmental contamination issues and using a lot of energy but it is biodegradation problem. In the process of purification, depolymerization, and separation a substantial amount of chemicals is used, resulting in toxic compounds that are harmful and difficult to degrade. Given PET's resistance, it is expected that a portion of this plastic stream will remain in landfills or the environment for hundreds of years (Austin *et al*., 2018).

## DOUBLE-EDGED SWORD

A PET-based plastic's durability can be attributed to its molecule over time, desirable versatility turned into a double-edged sword. Due to its plasticity and inertness, it poses a great challenge to recycle or degrade due to its industrial usefulness. Numerous approaches have been implemented in the past few decades to reduce plastic waste's deleterious effects. Since it can offer a "green route" for recycling PET and has several benefits, including ease of use, environmental friendliness, energy savings, and waste reduction, biological recycling has received a lot of attention recently (Chen *et al*., 2020). These consist of advertising using various bioremediation techniques and biodegradable polymers. There have been numerous attempts to create a successful bioremediation plan to degrade the rising amounts of plastic garbage. This has been studied in relation to numerous kinds of plants, bacteria, algae, fungus, and archaea. These organisms have shown variable degrees of success in handling the various hazardous wastes and are known to produce a variety of enzymes, including laccases, peroxidase, lipases, and hydrolases. Since these bioremediation processes add hardly any harmful chemicals to the environment, they are a considerably safer and more economical alternative to conventional waste management technique (Joutey *et al*., 2013; Weiland., 2020).

## EMERGING ENZYMES WITH BIODEGRADATION ABILITIES

Only microbial breakdown of polyethylene has been carried out globally, resulting in a 30-day degradation of about 20% (Bombelli *et al*., 2017). Successful, it was found that quick biodegradation of PE produced ethylene glycol. More than 24 distinct enzymes that can break down PET have been found thus far. The hydrolysis of the PET polymer into TPA, EG, BHET, and mono-(2-hydroxyehyl) terephthalic acid (MHET) is facilitated by each of these enzymes, which are esterases, hydrolases, and lipases. (Wilkes and Aristilde, 2017). Bacteria secrete extracellular enzymes that depolymerize PET outside of their cells in order to produce water-soluble intermediates. The microbes can then take those tiny molecules and use them for additional digestion and metabolism. In addition, enzymes produced by a number of species and strains create biofilms on the surface of the plastic.

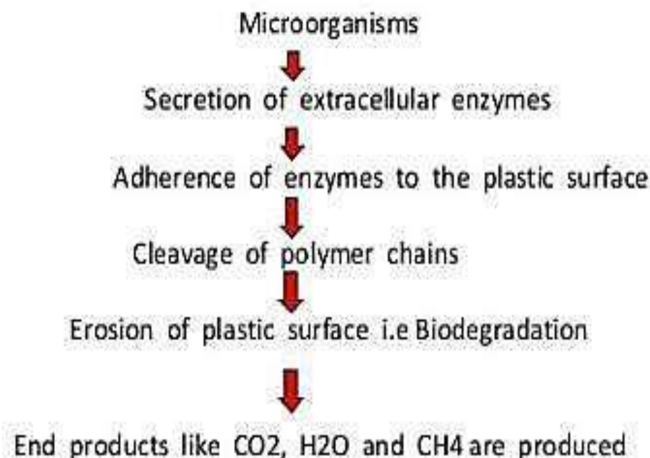


**Fig.2.** Bacterial enzymatic biodegradation mechanism.

Special enzymes are secreted like PHA depolymerase, polyurethenase, PEG dehydrogenase, and alkane hydroxylase to degrade polythene-based polymers. As per to the CATH database, the majority of PET degrading enzymes also belonged to the 3.40.50.1820 subfamily since they have a unique catalytic domain and adopt the standard alpha/beta hydrolase shape (Knudsen and Wiuf, 2010; Sousa *et al*., 2015. Approximately 15 distinct bacterial species, only a few species of algae *Chlamydomonas reinhardtii, Monoraphidium braunii*, etc., fungi (*Tramates versicolor, Pleurotus eryngii, Rhizopus, Phanerochacte chryososporium* and *Aspergillus*) that have been found to produce enzymes that can break down PET (Awasthi *et al*., 2017).

**Table 1.** There have been several microbial consortia evaluated over time for their capacity to digest plastic wastes, with varying degrees of effectiveness.

| Organism | Enzyme | Discovery | Amino acid residue | Reference |
|---|---|---|---|---|
| *Ideonella sakaiensis* | PETase | 2016 | 290aa | Yoshida *et al*., 2016 |
| *Ideonella sakaiensis* | IsMHETase | 2016 | 240aa | Yoshida *et al*., 2016 |
| *Pseudomonas Aestusnigri* | PaPETase | 2017 | 304aa | Bollinger *et al*., 2020 |
| *Leaf-branch compost* | LCC | 2011 | 258a | Sulaiman *et al*., 2012 |
| *Thermomonospora fusca* | TfHCut | 1998 | 261aa | Chen *et al*., 2008 |
| *Saccharomonospora viridis* | SvCut190 | 2014 | 304aa | Pati *et al*., 2009 |
| *Fusarium oxysporum* | FoCut5a | 2015 | 230aa | Dimarogona *et al*., 2015 |
| *Candida Antarctica* | CaLipB | 1998 | 317aa | Stauch *et al*., 2015 |
| *Thermobifida fusca* | TfCa | 2010 | 497aa | Billig *et al*., 2010 |
| *Thermomyces lanuginosus* | TILip | 1889 | 269aa | Arima *et al*., 1972 |

## GENERNAL ASPECT OF IDEONELLA SAKAIENSIS

Ideonella sakaiensis 201-F6 was identified as a bacterial strain in 2016 by recycling facility in Japan,Sakai city (Joppa, 2016). Based on the sequencing of its 16S rRNA, the polyphasic taxonomic investigations have revealed that Ideonella sakaiensis is a novel species of the genus Ideonella (Tanasupawat *et al*., 2016). According to bacterial morphology it investigations, is a gram- negative non-spore-forming member of the Betaproteobacteria phylum, aerobic bacillus with a unipolar flagellum having complete margined non-pigmented round colonies (Hiraga *et al*., 2020; Maity *et al*., 2021). The bacteria is extremely sensitive to even the smallest changes in salinity or ideal temperature range (30–37°C), yet it can handle mildly acidic (pH 5.5) or alkaline (pH 9.0) environments, with neutral pH being the best for growth. Ideonella. sakaiensi that uses PET as its main carbon source was described by Yoshida *et al.* in 2016 and its morphologically differs from other strains when cultivated in the presence of PET films. They also create a biofilm-like structure on the surface of the plastic strips in addition to the planktonic forms. Through biological mechanisms, the biofilm's individual cells are physically attached to one another, establishing a network on the substrate. The bacterium is also attached to the PET film by a few tiny biological processes, which also deliver the enzymes needed to degrade the PET (Awasthi *et al*., 2017). Ideonella sakaiensis 201-F6 can not only degrade but also absorb it by ultimately producing $CO_2$ and $H_2O$.

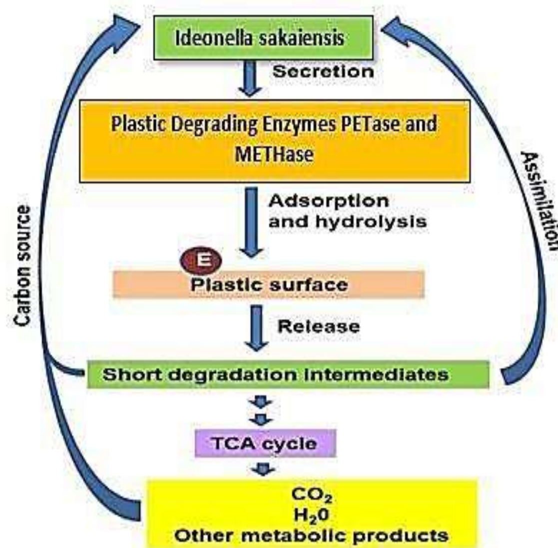


**Fig. 3.** The general process of Ideonella sakaiensis for biological breakdown of polymers in aerobic environments.

An open reading frame from this species that shares 23-51% of its genetic makeup with a previously found PET hydrolytic enzyme from the species Thermobifida fusca discovered through genetic investigation (Urbanek *et al*., 2021).

### PETase

PETase will now refer to the enzyme isolated from Ideonella sakaiensis. Decomposition is catalyzed by purified PETase, mono (2-hydroxyethyl) terephthalic acid MHET is the predominant product of the PET-to-MHET process, with bis (2-hydroxyethyl) terephthalic acid BHET being a secondly significant product, followed by ethylene glycol and terephthalic acid (TPA) (Graf *et al*., 2021).

### Structural Analysis of PETase

Initial PETase structure was determined by Han *et al.,* (2017). PETase has 290 amino acid residues and exists as a functional monomer. Several structural, experimental, and computational studies have investigated its three-dimensional structure (Joo *et al*., 2018). Understanding PETase structure may help with comprehension of the enzyme's mechanism. First, unlike other related enzymes, PETase contains two

disulfide bridges. To understand the PETase substrate -binding mode, high-resolution crystal structures are required since even minute variations between these enzymes are significant. All homologous structures must have the same disulfide bridge which connects the last helix and the C- terminal loop. PETase attaches to a wide, shallow, L-shaped, hydrophobic cleft that virtually spans the whole surface of the enzyme, allowing up to four polymeric residues to connect with the enzyme at once.

The enzyme displays a typical /-hydrolase fold made up of nine mixed-strands (some nonparallel and parallel), has seven -helices all around. PETase functions similarly to numerous other hydrolase enzymes, including lipases, esterase's, and proteases keeping the conserved triadic catalyst (Ser, His, Asp) (Wei and Zimmermann, 2017). According to the model, PETase's active site underwent a mutation to improve its affinity for polyester plastic and decrease steric hindrances surrounding it. This enhanced the capacity of PETase to bind and react with PET more efficiently. The G-X1-S-X2-G sequence, which has been found in the TfH, TfCut2, and LCC, is well conserved in the active site of cutinases and lipases (Bidegain and Paul-Pont, 2018). Previous research identified the catalytic amino acids and active site of PETase study. In PETase, the oxyanion hole is made up of Y58 and M132 based on its three- dimensional structure. S131, H208, and D177 made up the catalytic triad of PETase, according to a comparison of its amino acid sequence with that of TfCut2. As a nucleophile, S131 cleaves the ester bond, and Y58, containing aromatic groups, binds the substrate. In addition to W130 and W156, which are situated inside the substrate channel, these two molecules also take part in the binding process.

**Fig. 4.** 1. During the Catalytic Triad (light orange) cycle of PETase,S131 acts as a nucleophile and is polarized by H208 as it is in hydrogen bonding distance from H208. H208 is a base, D177 is an acid, and S131 acts as a nucleophile and is polarized by D177. The hydrogen bond between H208 and D177 stabilizes it. In HEMT (analogue of PET), S131 is also in hydrogen bonding proximity to the carbonyl atom (dark purple color). 2. In Y58 (swamp green) and M132 (swamp green), the NH groups form the oxyanion hole. Through the stabilization of carbonyl oxygen of HEMT (analogue of PET), M132 and Y58 stabilize the intermediate reaction (light orange).3. A wobbling W156 (lilac) in PETase has not been seen in any other PET-hydrolysing enzyme, even though they are all also containing tryptophan at a different position.4. PETase features two pale pink disulfide bridges.DS1 is unique to the PETase enzyme, whereas DS2 is absolutely conserved. DS1 is essential because it connects two loops that, respectively, comprise the basic and acid catalytic triads, H208 and D177 (both light orange). 5. Several residues with hydrophobic properties, M132 (swamp green), I179 (lime green), and W156 (lilac) are interacted with by HEMT (a homologue of PET), which is dark purple.

The active site is surrounded by six amino acids were selected for mutation based on an examination of the structural components involved are A211, S209, L88,R61, and I179 interact with the substrate during

PETase-substrate interaction (Liu *et al*., 2018; Ma *et al*., 2018). All of these amino acids are involved in substrate binding. Based on molecular dynamics (MD) simulations and crystal structure studies, a model of polymer chain binding within PETase's active site was proposed (Fecker *et al*., 2018). To make room for it, mutation was carried out the amino acids near the active site are more hydrophobic, or the amino acids near the active site have better affinity for PET. A large active site cleft at the active site contains a conserved catalytic triad of Serine (Ser160), Histidine (His237), and Aspartate (Asp206) residues. At the beginning architecture shows that on position 4, Ser160 is inserted into a nucleophilic elbow with a steep turn conformation in the region between loops 8 and 6. In the loop area between positions 7 and 5, the catalytic Asp206 residue is located, while positions 8 and 6 are home to His237. Additionally, it was noted that two disulfide connections existed between Cys203 (the end of position 7) and Cys239 (the eighth and sixth loops), as well as between Cys273 (the top of position 7) and Cys289 (after position 9). The Cys203-Cys239 disulfide was found to be essential for preserving the integrity of the catalytic triad, despite the fact that these covalent links frequently serve largely as thermal stabilizers. On the other hand, the PETase-specific disulfide is situated close to the active site and links the 7 - 5 and 8 - 6 loops, which contain the catalytic acid (D177) and catalytic base (H208), respectively. The 8 - 6 loop in PETase is also three residues longer.

**Enzyme Substrate Model**

Shorter loops seen in similar enzymes may prevent the creation of a subsite that can bind PET, according to the theory put forward by this extended loop (Joo *et al*., 2018). PETase's active-site cleft appears to be significantly wider than that of the other PET hydrolases. The PETase substrate model binding location is depicted in. It is evident that PETase creates a lengthy, shallow Lshaped gap on the surface of its protein. PETase's enzyme activity depends on the hydrophobicity of its substrate-binding cleft, which is primarily. These unusual structural characteristics could be the reason PETase can successfully accept and break down highly crystalline substrate PET (Han *et al*., 2017; Liu *et al*., 2018). In order to maximize the likelihood that the active and substrate binding sites of PETase would be completely exposed when the surface display system was built, a flexible linker (GGGGSGGGGS) was employed to link the C terminal of PETase and N terminal of anchor proteins. The surface charge of is PETase is extremely polarized, generating a dipole across the macromolecule and an isoelectric point of 9.6 (Wu *et al*., 2018). With the use of genetic and biochemical research, the scientists were able to pinpoint the enzymes responsible for external PET hydrolysis and the subsequent intracellular pathway of PET -hydrolytic product catabolism. It is suggested that PETase attaches to and hydrolyzes PET through an action mechanism.

**MHETase**

MHETase, which is essential for complete PET breakdown, still has an unknown structure. PET is broken down by the enzyme PETase, which results in the major byproduct, mono(2hydroxyethyl) terephthalic acid, or MHET. It was discovered by Yoshida *et al* in 2016 that Ideonella cultures kept on PET films had only tiny amounts of MHET, indicating the presence of extra molecular machinery that might degrade MHET. Whereas MHETase is the second enzyme that hydrolyzes PET. It is an intracellular enzyme that has 603 amino acids and a molecular weight of 65 kDa. Further research revealed the existence of a second enzyme, known as MHET hydrolase or MHETase, that used MHET as a substrate(Yoshida *et al*., 2016)

**Structural Analysis of MHETase**

All known plastic-degrading enzymes exhibit the /-hydrolase fold. However, MHETase is probably going to have a structure that is unheard of for degrading enzymes for plastic. This could be taken advantage of in order to enhance catalysis, increase substrate specificity, and considerably speed up enzymatic breakdown of plastic polymers. Once more, MHETase in three different states: ligand-free (2.05 nm), bonded to benzoic acid or a mono-(2-hydroxyethyl) terephthalamide that cannot be hydrolyzed, and ligand-free PETase (2.0 nm 19 It is believed that the MHETase's N-terminal amino acid sequence, also known as the "tether," which consists of residues 1 through 25, serves as a flexible linker between the rest of the protein and the bacterial outer bilipid layer, where the lipidized Cys residue is inserted.

MHETase attached to benzoic acid or a substrate analogue that cannot be hydrolyzed. Even substrate sub-structures and analogs, like benzoic acid or nicotinic acid, can bind to MHETase tightly in addition to the whole substrate, as shown in the corresponding costructure and in differential scanning fluorimetry (DSF) experiments. In experiments, it was discovered that MHETase solely hydrolyzes MHET and does not process other tannase enzymes' products, such as p-nitrophenyl compounds, BHET, aliphatic esters, or

aromatic esters like ethyl gallate and ethyl ferulate. It indicates that the substrate specificity is fairly constrained. Both of these enzymes share numerous characteristics related to the catalytic domain and are members of the cutinase family.

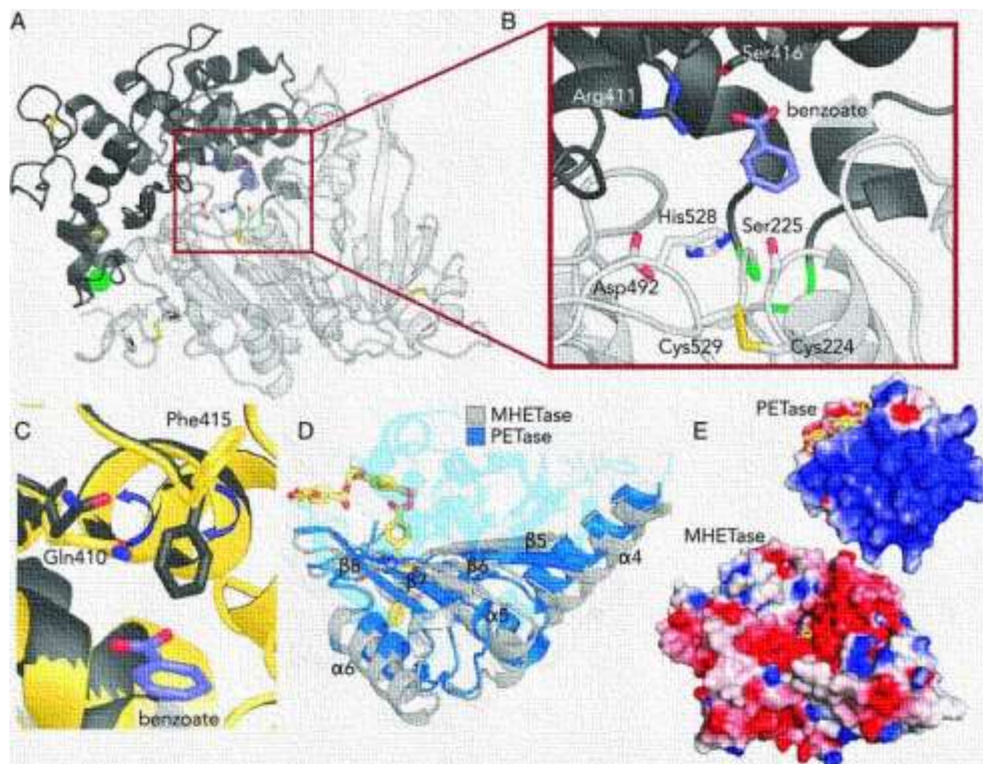


**Fig. 5.** (A) The MHETase structure (1.6 resolution, PDB ID code 6QZ3) with the catalytic triad, five disulfides (represented by yellow and grey sticks), benzoate (represented by purple sticks), and calcium ion (represented by a green sphere) highlighted. In contrast to the hydrolase domain, the lid domain is a light grey colour. Lime green main-chain atoms can be seen at Tyr252 and Ala469 of the linking residues (also in B). (B) An up-close view of the MHETase active site with benzoate bound; the catalytic triad of the active site disulfide, Ser416 and Arg411 is represented by sticks. (C) Purple arrows show how Gln410 and Phe415 are coordinated to move to the ligand-bound state when the apo enzyme (yellow) is superposed over the ligand-bound state (grey). Benzoic acid is represented in purple relative to other compounds. MHETase (grey) and PETase (blue) share similar domains in the hydrolase domain, as shown in (D). An PET tetramer from a previous docking research, is presented in yellow sticks. MHETase (29) and PETase (29), depicted by coloured gradients, demonstrate electrostatic potential distributions on their solventaccessible surfaces. In the PETase display, we see a bound PET tetramer, while in the MHETase display.

**Enzyme Substrate Model**

Unexpectedly, the disulfide bridge (Cys224-Cys529) that connects the loops containing the catalytic Ser and His residues is conserved among the members of the tannase family (Knott *et al*., 2020). The SerHis-Asp-Catalytic Triad is located in the active region of both enzymes, which is flanked by a "lipase box". The MHETase catalytic triad is arranged in a very compact manner thanks to an extra disulfide link between C224 and C529 that effectively brings the Ser and His residues closer to one another. The tannase/esterase family of enzymes, which is a member of Block X of the /-hydrolase fold enzymes listed in the ESTHER database and are closest fungal homologs. MHETase share a number of structural features studies on the IsMHETase structure demonstrate that it is a monomer, with the a lid domain and a catalytic hydrolase fold are the two different domains of MHETase (Sagong *et al*., 2020). According to the ESTHER database, this enzyme is classified as a /-hydrolase fold and is a member of Block X of the tannase family. Although the catalytic triad and oxyanion hole residues are a part of the /-hydrolase domain, the lid domain is nearly entirely responsible for substrate specificity. The phenyl ring of MHETA makes hydrophobic

interactions with the /-hydrolase domain predominantly at F495, and to a lesser extent at G132 and A494. Surprisingly, the lid domain residues F415, L254, and W397 strongly bind to almost the whole MHETA phenyl moiety. MHETase's two domains, together with an oxyanion hole created by the amine groups on the backbones of residues Gly132 and Glu226.(CF *et al*., 2021) Its catalytic domain shares a great deal of structural similarity with that of FaeB from Aspergillus oryzae and has high sequence similarity with homologues from Hydrogenophaga sp and Comamonas thiooxydans.

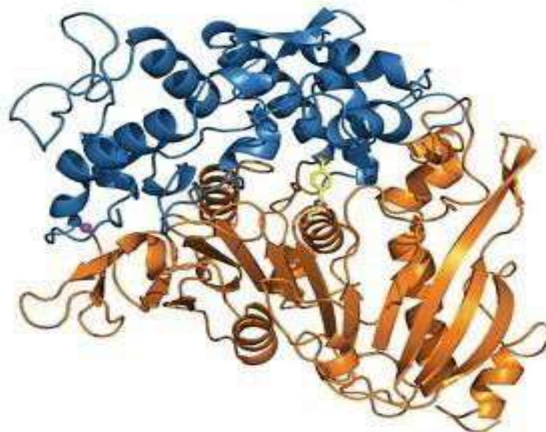

**Fig. 6.** The hydrolase domain (orange) and lid (blue) of MHETase act together to separate MHET (yellow).

The lid domain residues are primarily responsible for creating the interactions with benzoic acid and positioning it in the same location as MHETA. Mutations were employed to map the active site's structure, and the results of binding tests with different substrates were used to guide the generation of MHETase variants with higher product inhibition resistance activity towards MHET or even altered substrate specificity towards BHET. The lid domain of IsMHETase contains approximately 240 amino acids. $Ca^{2+}$ binding sites were located in the lid domain of MHETase, indicating that the enzyme shares structural similarities with the feruloyl esterases (Palm *et al*., 2019). It also suggests that the binding of $Ca^{2+}$ is crucial for the stabilisation of the lid structure. Although there is a striking structural similarity between MHETase, the lid domain of MHETase includes a number of extra loops that give it an unmatched substrate selectivity.The hallmark - hydrolase fold, a distinctive feature of the esterase family, makes up the active site of MHETase. The PETase and MHETase enzymes share a striking degree of resemblance in their core catalytic domains, according to high-resolution crystallographic analyses.

**FUTURE PERSPECTIVE**

The understanding of sustainability has grown over the past few decades in both the production and recycling of plastic materials. Sadly, the characteristics that high durability and low cost, among other factors that made them easy to use, hampered the creation of waste management procedures that were competitively priced. (Tournier *et al*., 2020) The discovery of the metabolic pathway using the enzymes PETase and MHETase representing a significant step forward in developing a bio-recycling plan for synthetic polymers. The ability to manufacture value-added goods like organic acid, fatty acids, enzymes, and others using genetically modified microorganisms will likely be available in the future. Genetically modified microbes offer a possible replacement for the plastic reusable economy, as this review has highlighted. The structure, purpose, and mechanism of the IsPETase and MHETase are currently the subject of considerable research. Furthermore, new studies on this enzyme keep coming out as the demand for alternatives that can degrade PET increases. Many research has produced X-ray structures of both enzymes with various mutations and bound molecules, and they have postulated what certain traits may have contributed to the enhanced enzyme activity. Genetically modified microbes offer a possible replacement for the plastic reusable economy, as this review has highlighted. There are still a lot of problems that need to be solved, but it is likely that PETase will be used in a lot more applications. For the identification of future similar enzymes, a fuller comprehension of molecular mechanism will be an excellent place to start (Danso *et al*., 2018).

## CONCLUSION

The enzymatic breakdown of PET and other polymers is becoming more significant and interesting to researchers as plastic output continues to rise. This method of plastic degradation is regarded as an innovative, ecologically beneficial approach to recycling post-consumer plastic products. Therefore, the use of genetic engineering may be essential to finding a solution to the plastic pollution issue in order to adapt the enzymes to synthetic polymers. However, the development of novel hydrolases with highly effective and focused catalytic properties. Also may assume that the highly adaptable enzymes released by I. sakaiensis will have a significant influence on how plastic materials are thought of in the future. Concern should also be expressed regarding the unrestricted patenting of plastic-degrading enzymes, which are crucial for waste cleanup internationally. Together, it can be claimed that PETase and MHETase provide distinct prospects for optimization as an effective biodegradation tool that can be used to effectively address the problem posed by plastic wastes.

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