

recycling plastic is



**NOT ENOUGH**

what if there was an

**ENZYME**

that could

**DIGEST  
PLASTIC WASTE**

and turn it into

**WATER**

# **Leaf-branch compost cutinase**

## **PETase**

## **MHETase**

**Scientists have turned to nature to help us break down plastic.**

**By using these enzymes, they've managed to turn plastic into water and carbon dioxide. Even though plastics are non-natural chemicals, many microorganisms are able to metabolize them.**

**Recently, we've seen incredible advances: scientists found ways of degrading PET (the material a plastic bottle is usually composed of) in just ten hours. The enzymes they've worked with is now 10,000 times more efficient than the initial one they tested, and resists high temperatures. This is a step closer to achieving real, infinite recycling of plastic bottles on a large scale.**

PET biodegradation has been extensively studied because esterase enzymes (enzymes that split esters into an acid and an alcohol) are abundant in nature<sup>2</sup>. Reports on the biological degradation of PET or its utilization to support microbial growth are, however, infrequent. Some organisms from the filamentous fungi group, *Fusarium oxysporum* and *Fusarium solani*, have been grown on a mineral medium containing PET yarns<sup>3</sup>.

In 2016, Yoshida et al<sup>4</sup> reported the discovery and characterization of the soil bacterium strain,  *Ideonella sakaiensis* 201-F6, found growing in PET-contaminated sediment near a plastic recycling facility in Japan. This gram-negative, aerobic, rod-shaped bacterium has the remarkable ability to use PET as its major carbon and energy source for growth.

*I.sakaiensis* employs a two-enzyme system to depolymerize PET to its building blocks, TPA and EG, which are further catabolized to a carbon and energy source. One of the two enzymes, ISF6\_4831 protein, hydrolyzes and breaks ester linkages. With a preference for aromatic rather than aliphatic esters, and a specific inclination towards PET, it is designated as a **PET hydrolase (PETase)**. The PETase enzyme in *I. sakaiensis* is a cutinase-like serine hydrolase that attacks the PET polymer, releasing **bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET)** and TPA. PETase further cleaves BHET to MHET and EG. The second enzyme, ISF6\_0224 protein, MHET hydrolase (MHETase), further hydrolyzes the soluble MHET to produce TPA and EG (Fig. 2). Both enzymes are required, likely synergistically, to enzymatically convert PET into its two environmentally benign monomers, TPA and EG4, making it possible to fully recycle PET.

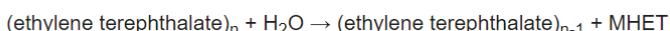
# Sources

In 2020, a major advance saw scientists identifying another enzyme that could degrade PET in just 10 hours<sup>10</sup>. The research screened a large variety of bacteria and enzymes for potential candidates, including the leaf-branch compost cutinase, LCC, that was first discovered in 2012. Hundreds of mutant PET hydrolase enzymes were then produced by varying amino acids at the binding site and improving thermal stability. Bacterial mutants were then screened to identify efficient PET decomposers. After running this process for multiple rounds, a mutant enzyme was isolated that is 10,000 times more efficient in degrading PET than the native LCC. It is also stable at 72°C, close to the melting temperature of PET. This finding contributes significantly towards attaining the infinite recycling of PET and is already at a pilot industrial stage<sup>10</sup>.

## PETase

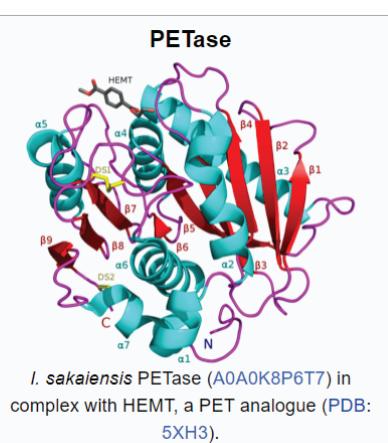
From Wikipedia, the free encyclopedia

**PETases** are an **esterase** class of enzymes that catalyze the hydrolysis of polyethylene terephthalate (PET) plastic to monomeric mono-2-hydroxyethyl terephthalate (MHET). The idealized chemical reaction is (where n is the number of monomers in the polymer chain):<sup>[1]</sup>



Trace amount of the PET breaks down to **bis(2-hydroxyethyl) terephthalate (BHET)**. PETases can also break down PEF-plastic (**polyethylene-2,5-furandicarboxylate**), which is a bioderived PET replacement. PETases can't catalyze the hydrolysis of **aliphatic polyesters** like **polybutylene succinate** or **polylactic acid**.<sup>[2]</sup>

Non-enzymatic natural degradation of PET will take hundreds of years, but PETases can degrade PET in matter of days.<sup>[3]</sup>



Degradation of plastics by microbial and/or enzymatic means ([Figure 2](#)) is a promising strategy to depolymerize waste petro-plastics into monomers for recycling, or mineralize them into carbon dioxide, water, and new biomass, with concomitant production of higher-value bioproducts ([Grima et al., 2000](#); [Montazer et al., 2019, 2020a](#)). Biodegradation of plastics involves excretion of extracellular enzymes by the microorganism, attachment of enzyme to the surface of plastic, hydrolysis to short polymer intermediates, which are ultimately assimilated by microbial cells as carbon source to release CO<sub>2</sub>. Despite the fact that these plastics represent non-natural chemicals, several microorganisms capable of metabolizing these polymers have been identified in recent years. Over 90 microorganisms, including bacteria and fungi, have been known to degrade petroleum-based plastics ([Jumaah, 2017](#)) mostly *in vitro* condition.

To achieve depolymerization, scientists have looked to nature, searching for microbial enzymes that can break down plastics. In 2012, researchers at Osaka University discovered an enzyme in a compost heap that can break down one of the world's most used plastics: **Polyethylene terephthalate (PET)**, CAS Registry Number 25038-59-9, formula (C<sub>10</sub>H<sub>8</sub>O<sub>4</sub>)<sub>n</sub>.

The enzyme, known as **leaf-branch compost cutinase (LLC)**, breaks the bonds between PET monomers, but it is intolerant to the 65°C softening temperature of PET, [denaturing after a few days of working at this temperature and limiting its industrial practicability.](#) Since depolymerization can only take place in molten plastic, enzymes must be stable at increased temperatures

## Characterization and engineering of a two-enzyme system for plastics depolymerization

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