Discovery of a novel PETase-like enzyme for the degradation of plastic waste

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Background

Since the beginning of the large-scale production of plastics in the 1950s, these materials have found a wide variety of applications and became essential in today's society. The annual production of plastics worldwide was estimated in 2017 to be 348 Mt, 10% of which was produced from renewable sources. Almost half of this plastic is channelled to the packaging sector, which contributes greatly to municipal solid waste and marine litter. Abandoned plastic waste poses an enormous environmental problem, contaminating the soil and oceans. Polyethylene terephthalate (PET) is synthesized by chain polymerization of terephthalic acid and ethylene glycol. Its properties (durability, low price and convenient processability) made PET the main material for bottles production adopted by the beverage industry. Even though PET has the highest collection rates compared to other plastics, in 2017 only 29% of new PET bottles were made from recycled material.

Lately, new approaches for recycling of plastics have been proposed, incorporating microorganisms and their enzymes for depolymerization of used plastics and synthesis on new ones.

Studies dealing the enzymatic degradation of polyesters have been performed for over 15 years. However, since 2016 the discussion about PET-degrading enzymes has bloomed. The turning point was the work of Yoshida *et al.* (2016), who discovered a PET-assimilating bacterium, namely *Ideonella sakaiensis*. The enzyme responsible for the degradation of the polymer was identified and characterized as a PETase. Till then, enzymes belonging to the family of cutinases had been studied for PET degradation and especially cutinases from the thermophilic actynomycete bacteria of the genus *Thermobifida*. *Is*PETase shares 45–53% amino acid sequence identity with the actinomycete cutinases.

MoPE can degrade various crystalline polyesters

Polymer	Dry mass decrease (%)	Molecular weight decrease (M_n%)
Polycaprolactone (PCL)	**33,4 ± 1,0**	**10,5 ± 3,0**
Polylactic acid (PLA)	0.0 ± 0.0	**10,3 ± 2,2**
Polybutylene succinate (PBS)	5,3 ± 1,1	Not measured
Polyhydroxybutyrate (PHB)	8,3 ± 2,9	Not measured
Polyurethane (PU)	3,6 ± 1,2	4,1 ± 1,8

The novel enzyme can be considered a **polyesterase** as it can degrade different types of polyesters. Its highest activity was shown on polycaprolactone, achieving the impressive 33% weight loss and 10.5% reduction in M_n. A similar M_n decrease was also observed on PLA. For the rest of the polymers, low degradation efficiencies were observed.

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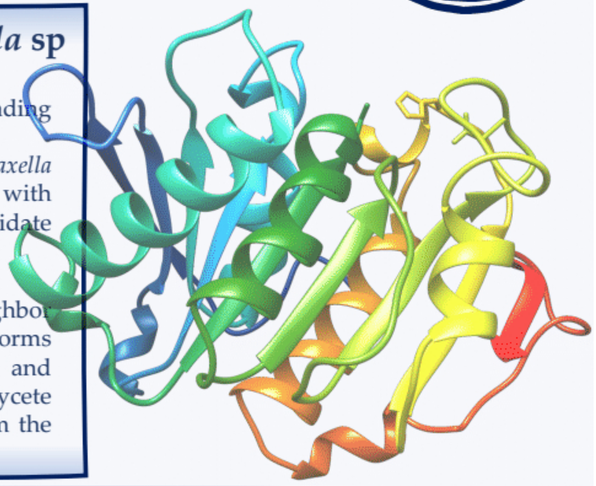
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 870292.

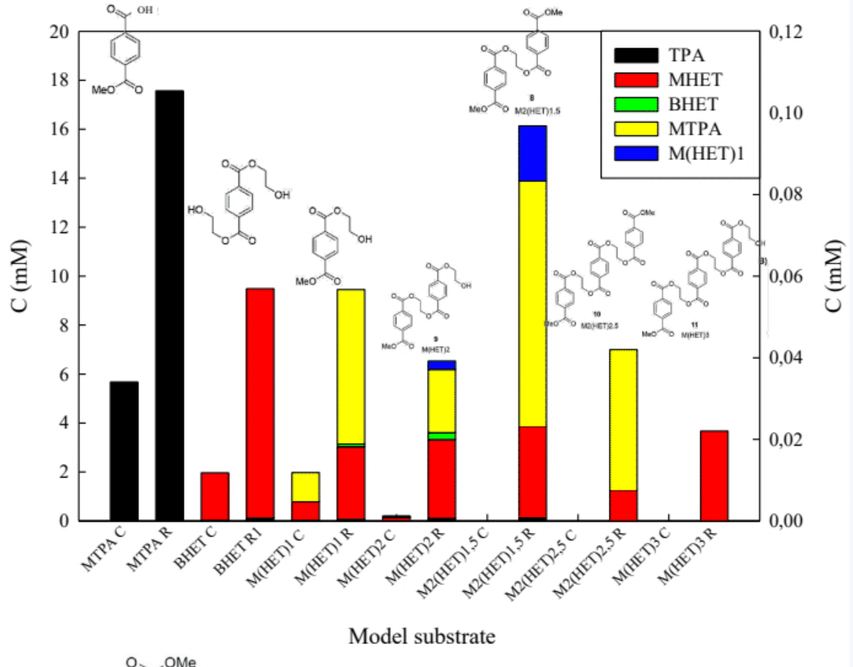
Identification of a novel PETase (MoPE) from Antarctic bacterium *Moraxella* sp

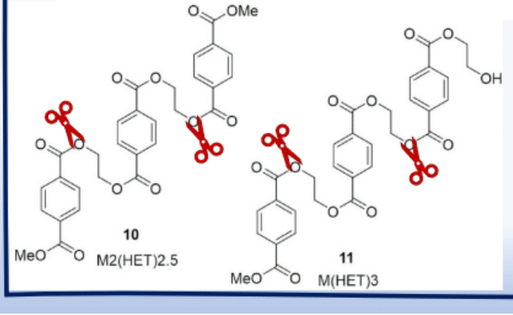
Homologous protein sequences of known PET-degrading enzymes were identified using BLAST algorithm.
A lipase-type enzyme from Antarctic bacterium *Moraxella* sp. showed high sequence homology (42-46%) with IsPETase and other cutinases, being a promising candidate for polyesterase activity.

A phylogenetic tree was constructed using the neighbor Joining method showing that the *Moraxella* sequence forms a separate branch that is closest to IsPETase and *Pseudomonas aestusnigri* polyesterase, while the ascomycete cutinases are grouped together with the cutinase from the leaf-and-branch compost metagenome (LCC).



Mechanism for PET oligomer degradation

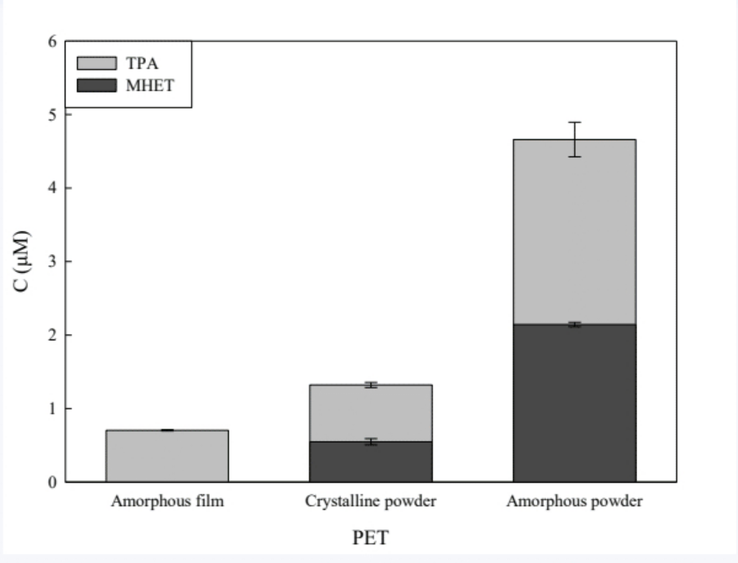




MoPE could hydrolyze **PET oligomers** cleaving from the sides recognizing an MHET moiety. Methyl TPA is also recognized in a similar way as MHET.

On the other hand, the activity of the enzyme on MHET is very low (similar to IsPETase).

MoPE can degrade crystalline and amorphous PET



MoPE's activity was tested on **3 PET materials**, amorphous film (0.66 mm thickness), amorphous powder and powder of *35% crystallinity* (<500 μm diameter).

As a rule, crystallinity negatively affects the activity of depolymerases.
However, MoPE showed the lowest activity on amorphous films compared to both powdered materials, probably due to the much lower surface area.

The product release from amorphous powder was 3.5–fold higher than from the crystalline material.