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

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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. While in the 1960s, the percentage of plastic waste in municipal solid waste was less than 1%, this number increased to around 10% in 2005 in countries with middle/high income (Almeida *et al.*, 2019). 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 (Wei *et al.*, 2019). Abandoned plastic waste poses an enormous environmental problem, contaminating the soil and oceans. This is due to microplastic particles, which are released through natural weathering phenomena. These microplastics are ingested by animals and end up in humans through the food webs (Son *et al.*, 2019).

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 (Salvador *et al.*, 2019). Recent studies have highlighted the failure of traditional recycling processes for plastics, as only 9% of virgin plastic produced out of 8 billion metric tons of oil was recycled (Salvador *et al.*, 2019). 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.

The present work aimed to the discovery of a novel PET-degrading enzyme, using bioinformatics tools. The few known PET-degrading enzymes were used as templates for the identification of homologous sequences. The search led to a protein sequence originating from an Antarctic psychrotrophic lipolytic bacterium of the genus *Moraxella*. The sequence has been designated as a triacylglycerol lipase and shares the highest similarity with IsPETase (45.2%), followed by the actinomycete cutinases (41.48-46.05%). A synthetic gene was constructed, optimized for expression in *Escherichia coli* and cloned in pET22b(+) vector. The native signal peptide of the protein was excluded from the gene. Expression took place in *E. coli* BL21 (DE3) after induction with 0.1 mM IPTG at 18 °C for 20 h. The recombinant protein with an apparent molecular mass of 33 kDa was functionally expressed in this system and purified using immobilized metal affinity chromatography (IMAC). The enzyme was biochemically characterized and its ability to degrade PET model substrates, various PET materials (crystalline and amorphous powders and films) and other polyesteric plastics was tested. The pure enzyme could degrade PET powders more efficiently than the film. It also showed very high degradation ability on polycaprolactone (PCL) and could also degrade polylactic acid (PLA). The degradation efficiency was assessed though weight loss measurements, polymer molecular weight reduction and product detection.

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