**High- throughput screening assay for the discovery of novel PETases**

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**ABSTRACT**

Global demand for polymers has rapidly grown over the last decade, but more than 40 % of total plastic production ends up in the environment, burdening it significantly. Step-growth polymerization of terephthalic acid and ethylene glycol results in synthesis of polyethylene terephthalate (PET), whose barrier properties have made it the main material for bottle production adopted by the beverage industry. Despite the fact that most people are aware of recycling benefits and PET recycling rates are quite high for some European countries, still in other cases PET bottle waste ending up in landfills remains above 50 %. Biodegradation of discarded PET packages, can take up to centuries, as PET is an extremely persistant polymer in biological and microbial degradation. The enzymatic degradation of PET can be a promising alternative as it allows the re-polymerization of monomers and the production of plastics with similar properties to the starting material. The discovery of novel PET-degrading enzymes can be a time- and source-consuming process, as it requires several experiments including the enzymatic treatment and the determination of polymer properties. In this study, we developed a new screening method, in which labeled PET model substrates were synthesized and enzymatically hydrolysed by different benchmark and potential PET-degrading enzymes. Qualitive and quantitive studies confirmed that the enzymes with high depolymerizing activity on PET were also active on model substrates, a fact which allows fast and direct detection of PETase-like enzymes. High- throughput screening assay could precisely predict enzyme activity on PET as it was absolutely related with tests on PET powder, offering an important tool for the discovery of novel enzymes, which can be a promising upcycling alternative towards plastic waste valorization.

**KEYWORDS:** high- throughput screening, polyethylene terepthalate, enzyme, biodegradation