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# Novel fluorescent compounds for screening and characterizing PET-degrading enzymes

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## PURPOSE OF THE ABSTRACT

Poly(ethylene terephthalate) (PET) is one of the most commonly used polymer resins, especially in the food and beverage industry, as it has great barrier properties, excellent chemical resistance, and stability over a broad temperature range. Even though most people are aware of recycling benefits and PET recycling rates are quite high for some European countries, in other cases, PET bottle waste ending up in landfills remains above 50%.

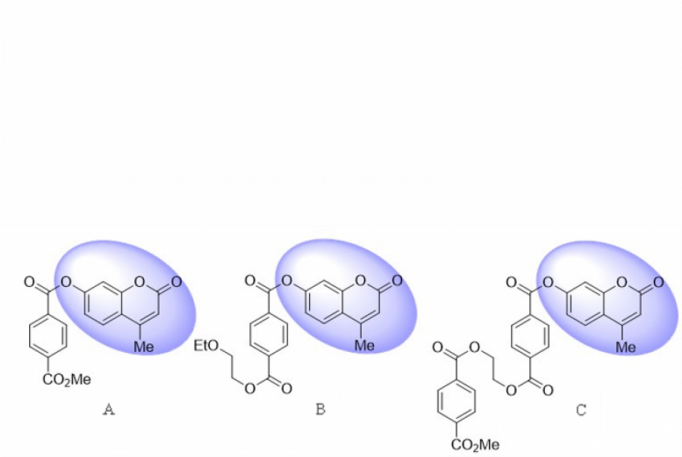
Traditional recycling after mechanical and chemical treatment can completely convert PET waste into new products, although the resulting recycled polymer is of lower quality due to mechanical stress and oxidation during the process [1]–[3]. Regarding chemical treatment, this alternative requires high temperatures in conjunction with toxic chemicals, making the process cost-effective and energy-intensive [4]. On the other hand, enzymatic degradation of PET is highly dependent on material properties such as crystallinity [5], but still, it is the most eco-friendly alternative for handling PET debris, as it requires mild reaction conditions, reducing the energy and reagent consumption [6]. Although the development of new polyester hydrolases that act on highly crystalline PET materials remains a challenge, the enzymatic hydrolysis of PET is a process that is ever-improving [7].

The main enzymes involved in PET degradation are carboxylesterases, cutinases, and lipases [8]. Discovering novel PET-degrading enzymes can be a time-consuming and resource-intensive process. This involves screening metagenomic libraries using appropriate substrates or following conventional methods of microorganism isolation and cultivation under optimal conditions [9]. Directed enzyme evolution and semi-rational strategies comprise other sources for discovering novel PETases, although the major impediment in this approach is the absence of efficient high-throughput screening [9], [10]. A usual approach followed by several reports often includes short (C2-C6), medium (C8-C10), or long-chain (>C10) p-nitrophenyl acyl esters (p-NP esters), which aim to predict and/or compare putative PET hydrolyzing enzymes [11]–[13]. Nonetheless, in that case, no correlation between esterase and depolymerizing activity should be taken into consideration [14], as PET polymer structure substantially differs from these substrates. Alternative approaches perform colorimetric, spectrophotometric, and fluorometric high-throughput assays using substrates, such as synthetic PET oligomers, or PET powder in suspension [17]–[21], although these techniques are often subjected to experimental restrictions such as the use of highly corrosive solvents or the need for secondary analyses for the quantification of released products.

In this work, novel fluorescent compounds were synthesized in order to screen and characterize PET-degrading enzymes. In more detail, the structural design of these compounds mimics the structure of PET oligomers and contains a fluorescent moiety (Figure 1), whose release allows real-time monitoring of enzyme activity. The performance of these substrates was evaluated after conducting kinetic studies using various polyesterases

including LCC-ICCG variant, IsPETase from Ideonella sakaiensis, and MoPE from Moraxella sp., as well as three enzymes that are not considered PET hydrolyses and cannot degrade PET. The kinetic parameters were further correlated with the ability of the enzymes to degrade virgin PET, proving that some of these PET model substrates can be utilized not only for a rapid, and sensitive high-throughput screening of novel PET-degrading enzymes but also in evaluating the performance of the enzymes that have been mutated using protein engineering tools.

## FIGURES




## FIGURE 1

Figure 1

Structure of model substrate (A) mUPET1, (B) mUPET2 and (C) mUPET3. The fluorogenic moieties, which are released due to the enzymatic hydrolysis, are depicted in blue circles.

## FIGURE 2


## KEYWORDS

enzyme screening | polyethylene terephthalate | fluorescent substrates | plastic degradation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