Towards novel PET-ases: Whole-cell biotransformation of PET-related substrates

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With more than 350 million tons of plastic being produced annually and a 3% annual increase in production, plastic waste has become a global environmental and health problem. Polyethylene terephthalate (PET) is a ubiquitous plastic and is the most commonly used packaging material (indicated by the recycling code '1') and used in the manufacture of 60% of fast fashion clothing. While post-consumer PET beverage bottles are highly commercially valuable, due to the high purity grade PET content, mixed multi-layered PET packaging, pots, tubs and trays as well as textiles are largely unrecyclable and present a considerable waste burden. Post use, only 9% of all plastic ever produced was recycled, the majority ends up in landfills, incinerators or the environment. Research in recent years has identified many enzymes with the potential to degrade plastic, however, only a handful are efficient enough to be used on an industrial scale. Advances in protein engineering of mentioned enzymes have largely improved their activity but a need for discovering new enzymes remains. In this work, we aimed to identify novel plastic degrading biocatalysts by screening novel microorganisms and employing a set of specific PET-related substrates, including PET- dimer and trimer.

A total of 251 microorganisms were isolated from contaminated and uncontaminated sites using four different growth media, in order to promote the diversity of isolated microorganisms. All of the isolated strains were screened for their potential to degrade synthetic polymers. The screening was carried out on Mineral Salt Medium (MSM) agar plates with various plastic polymers and monomers as the sole carbon source. Strains able to produce clearing halos on such plates were selected for further experiments. Seven strains produced clearing halos on bis(2-hydroxyethyl) terephthalate (BHET) a monomer of polyethylene terephthalate (PET).

In order to gain access to potentially novel PET hydrolases, eight PET-related substrates were used for biotransformation reactions in a whole-cell biocatalytic system. Three commercially available substrates (phthalic acid, terephthalic acid and BHET) and five newly synthesized PET-related esters named: M(HET)1, M2(HET)1,5, M(HET)2, M2(HET)2,5, M(HET)3 (Djapovic, et al 2021). Reactions were carried out with resting cells in MSM medium, incubated at 37°C for 72 h and monitored chromatographically with products characterized by NMR.

New biocatalysts showed markedly different patterns of cleaving. Some strains were able to hydrolyze terminal ethylene glycol moieties from a range of substrates and couldn't hydrolyze terminal methyl moieties. However, when M(HET)2 (PET dimer) was used as a substrate, some biocatalysts were able to specifically hydrolyze internal ester bonds.

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