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Side to side comparison of a novel wild-type polyesterase from Deinococcus maricopensis with LCC-ICCG indicates promising degradation of semi-crystalline post-consumer PET

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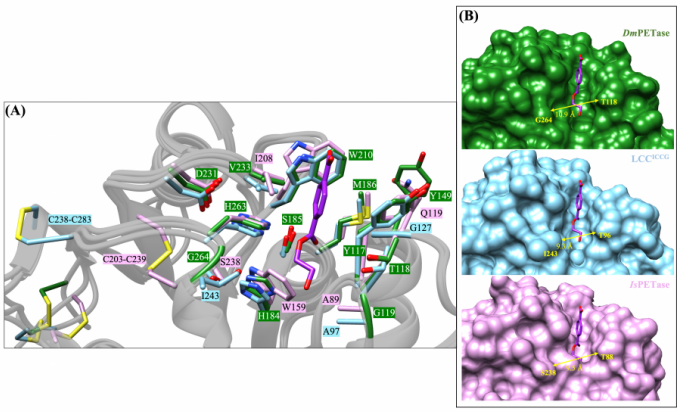
PURPOSE OF THE ABSTRACT

Synthetic plastics have become an integral part of modern life due to their versatile applications. However, their increased usage has resulted in a corresponding rise in waste generation, as their durability results in accumulation. Globally, according to recent studies, plastic waste primarily consists of polyesters, following the polyolefins, with PET being the most produced polyester resin in 2021 [1,2]. The majority of PET waste ends up in landfills, ultimately making its way into terrestrial and marine environments, as it presents low susceptibility in biodegradation because of the presence of aromatic components in its backbone [3–6]. Aiming to the hydrolysis of PET ester bonds, resulting to its degradation, recent advancements have led to the discovery of novel PET-degrading enzymes, such as the metagenome-derived LC-Cutinase (LCC) and its thermostable variant, LCC-ICCG, which demonstrates remarkable efficiency in depolymerizing amorphized PET waste [7,8]. As a result, LCC-ICCG is the only enzyme industrialized for PET biorecycling [9]. Concerning high crystallinity PET, it seems that LCC-ICCG presents room for improvement, as it follows the main trend of most well-known PETases whose effectiveness in PET degradation is significantly limited at increased crystallinity grades [10,11].

Hereby, we report the discovery of DmPETase, a novel wild-type polyesterase from the bacterium Deinococcus maricopensis, which depolymerase activity was tested on a broad range of synthetic materials, including PET and PU, as well as biodegradable polymers. Emphasizing on PET, our research focused on the effect of crystallinity on its degradation. Enzymatic reactions, with DmPETase and LCC ICCG, were conducted on virgin, amorphous, and semi-crystalline PET powder, as well as two types of post-consumer water bottles in their original and powdered form.

DmPETase's unique characteristics form its own sub-branch on a phylogenetic tree of characterized PET-degrading enzymes, while it displays a dissimilar electrostatic surface to well-known benchmark PETases (Figure 1). Its biochemical characterization depicted a thermostable cutinase-like enzyme with the ability to degrade various synthetic polymers, mainly PCL and PBS. Notably, DmPETase was proved capable of degrading crystalline compartments of PET bottles, as well as semi-crystalline PET powder at the same level as LCC-ICCG , while getting less affected by PET crystallinity grade (Figure 2). These novel findings demonstrate the potential of DmPETase as a promising enzymatic platform for protein engineering, specifically aiming in enhancing PET degradation rate through modifications to its active site, given the enzyme's natural high affinity to semi-crystalline PET.

FIGURES



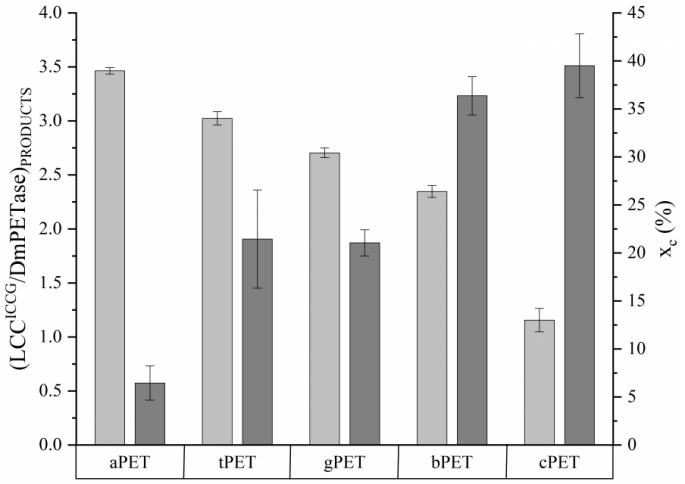


FIGURE 1

(A) Superimposition of DmPETase (green) with LCC-ICCG (blue-PDB ID 7VVE) and IsPETase (pink-PDB ID 6EQH), highlighting important residues participating in structural features (cysteines for disulfide bonds) or catalytic and substrate binding. Ligand 2-hy

FIGURE 2

Ratio of hydrolysis products released by LCC-ICCG to DmPETase (light grey) of PET powders of variable crystallinities (dark grey). PET powders are: aPET-amorphous PET of Xc 5 %, tPET-transparent PET bottle of Xc 21 %, gPET-green PET bottle of Xc 21 %, cP

KEYWORDS

PET degradation | PETase | novel polyesterase | Deinococcus maricopensis

BIBLIOGRAPHY

[1]R. Geyer, Sci. Adv. 3 (2017) e1700782
[2]PlasticEurope: Plastics - The Facts 2022, Plastics - the Facts 2022, (2022).
[3]L. Yang, Sci. Total Environ. 780 (2021) 146546
[4]P.E. Redondo-Hasselerharm, Sci. Adv. 6 (2020) eaay4054
[5]K. Cverenkárová, Life. 11 (2021) 1349
[6]Y.H.V. Soong, Bioengineering. 9 (2022) 98.
[7]V. Tournier, Nature. 580 (2020) 216-219
[8]S. Sulaiman, Appl. Environ. Microbiol. 78 (2012) 1556-1562.
[9]S. Thiyagarajan, RSC Adv. 12 (2022) 947-970
[10]V. Tournier, Chem. Rev. (2023)
[11]Z. Ding, J. Hazard. Mater. 453 (2023) 131386