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**ABSTRACT BOOK**



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# ***Engineering the activity of a thermophilic esterase from *Zhizhongheella caldifontis* for MHET degradation***

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Polyethylene terephthalate (PET) is the world's most extensively recycled polymer, the dominant material for beverage packaging, and valuable enough to drive sustained R&D into post-consumer recovery strategies.<sup>1</sup> Enzymatic depolymerization can fully convert PET into its monomers, terephthalic acid (TPA) and ethylene glycol (EG), enabling separation from mixed-plastic streams, upgrading to higher-value chemicals, and re-synthesis of virgin-quality PET, in contrast to the chain-degrading nature of conventional thermomechanical recycling.<sup>2,3</sup> Although various PET hydrolases (PETases) have been reported for this biorefinery route, their activity is inhibited by accumulation of the intermediate mono-(2-hydroxyethyl) terephthalate (MHET) and bis(2-hydroxyethyl) terephthalate (BHET).<sup>4</sup> Integrating an MHET hydrolase (MHETase), which converts MHET to TPA and EG, alleviates this bottleneck and restores full catalytic efficiency. Here, we report the engineering and characterization of a thermotolerant esterase from *Zhizhongheella caldifontis* (ZcEST) and its variants, including ZcMHETase (ZcEST\_D355N) and ZcBHETase (ZcEST\_D355S), which display up to 21-fold and 56-fold higher activity on MHET and BHET, respectively, compared to wild-type. High-performance liquid chromatography confirmed efficient conversion of PET-derived oligomers, while thermal shift assays and kinetic methods were employed to evaluate thermal stability and catalytic performance. These analyses reveal the structural basis for enhanced activity, substrate specificity, and thermal stability of the engineered variants. Our findings establish ZcEST-derived hydrolases as robust candidates for integration into enzymatic PET recycling processes, and highlight how rational enzyme design can guide the future of industrial biocatalysis, contributing to the development of efficient biocatalysts for circular plastic economies.

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## **Keywords**

Protein engineering; Substrate specificity; MHET hydrolysis; plastic depolymerization; PET recycling