Discovery of a thermotolerant MHET hydrolase scaffold using bioinformatics and machine learning

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ABSTRACT

Polyethylene terephthalate (PET) is the most recycled plastic worldwide, the most widely used material for beverage packaging, and valuable enough to solicit R&D projects to its after-use recovery [1]. Enzymatic depolymerization of PET to its monomeric units, i.e., terephthalic acid (TPA) and ethylene glycol (EG), allows for its separation from plastic mixtures, its valorization to valuable chemicals and the production of high-quality repolymerized PET, in contrast to material-degrading thermomechanical recycling methods [2, 3]. Several reported PET hydrolases (PETases) enable this waste−PET biorefinery, but suffer inhibitory effects due to the formation of monohydroxyethyl terephthalate (MHET); an intermediate hydrolysis product [4]. This inhibition can be rectified by introducing an MHET hydrolase enzyme (MHETase) that degrades MHET to TPA and EG [5]. However, the optimal temperature of enzymatic PET degradation is close to its glass transition temperature of 70 °C [6, 7], and while PETases and other esterases that can withstand these temperatures have been discovered and engineered before [8, 9], no reported MHETases can withstand temperatures higher than 50 °C [10]. In this work, putative thermotolerant MHETases are mined with the bioinformatics tools BLAST and machine learning-based ThermoProt [11, 12]. Selection is based on similarity to the well-studied *Is*MHETase from *Ideonella sakaiensis*, predicted protein secretion type and thermophilicity. The most promising candidate is *Zc*MHETase from *Zhizhongheella caldifontis* that is recombinantly expressed in *E. coli* Shuffle T7 and purified to homogeneity. Its temperature and pH stabilities and optima are experimentally determined on pNP−butyrate substrate, and its MHET hydrolysis capacity is compared to *Is*MHETase. Protein engineering of *Zc*MHETase aiming at enhancing catalytic activity is currently ongoing, based on previously identified *Is*MHETase hot-spot residues and inspection of relevant crystal structures [13]. Ultimately, *Zc*MHETase could be used as a new scaffold for designing more efficient enzymatic PET valorization and recycling systems.

KEYWORDS: MHETase, Thermotolerant, ThermoProt, Machine Learning, Bioinformatics

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