INVESTIGATION OF STRUCTURAL DETERMINANTS OF PLASTIC DEGRADING ENZYMES VIA X-RAY CRYSTALLOGRAPHY AND MOLECULAR DOCKING

P. Karampa[1], K. Makryniotis[2], E. Nikolaivits[2], P. Samoili[1], V. Daskalakis[3], E. Topakas[2], M. Dimarogona[1]*

[1]Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering, University of Patras, Patra, Greece

[2] Industrial Biotechnology & Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

[3]Laboratory of Biomolecular Dynamics and Engineering, Department of Chemical Engineering, University of Patras, Greece

(*mdimarog@chemeng.upatras.gr)

ABSTRACT

The enzymatic degradation of plastics is a promising solution for their uncontrolled accumulation on Earth [1]. Polyethylene terephthalate (PET) is one of the most common polymers used in packaging, construction, and agricultural industries [2]. Its semi-aromatic and semi-crystalline synthesis gives it high mechanical strength and barrier properties suitable for packaging [3]. Many enzymes that decompose plastic have been discovered since 2000, such as lipases and carboxyl ester hydrolases [3]. Most PET hydrolases (known as PETases) attack the polymer ester bonds leading to mono-(2-hydroxyethyl) terephthalate (MHET) as the main water-soluble degradation product. The MHET esterases (MHETases) cleave the ester bonds of MHET releasing terephthalic acid (TPA) and ethylene glycol (EG) as end products, making it possible to utilize them as feedstocks [4].

This study focuses on the investigation of structure-function relations of a serine hydrolase with potential degradation capacity against MHET. *Fo*FaeC is a feruloyl esterase (FAE) from the filamentous fungus *Fusarium oxysporum*. FAEs are enzymes of biotechnological interest since they cleave the ester bonds between hydroxycinnamic acids and arabinose in the plant cell wall, rendering it more vulnerable to enzymatic decomposition and releasing valuable compounds, such as ferulic acid. Interestingly, the search for structural homologs using the previously determined crystal structure of *Fo*FaeC (PDB code: 6FAT), showed that the third closest one is a bacterial MHETase from *Ideonella sakaiensis* (PDB code: 6JTT) [5,6,7]. In parallel, *Fo*FaeC activity on PET oligomers was experimentally verified. Recombinant *Fo*FaeC was expressed in *Pichia pastoris*, co-crystallized successfully with *p*-coumaric acid and the X-ray data were processed resulting in the first ligand-bound structure of a tannase-like FAE [6]. In the present work, we report the expression, crystallization, and structure determination of an *Fo*FaeC variant, G122S, that was created by structure-guided mutagenesis, in an effort to mimick MHETase active site. Compared to wild-type *Fo*FaeC, G122S variant exhibits increased catalytic activity against MHET. The crystallographic structure of both wild-type *Fo*FaeC and G122S variant were used for docking simulations aiming to acquire deeper understanding and interpretation of these biochemical findings. Shedding light on the structural determinants of PET-active enzymes will allow the production of robust biocatalysts for plastic degradation.

KEYWORDS: plastic degradation, MHETase, feruloyl esterase, X-ray crystallography, docking

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