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

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Introduction

Polyethylene terephthalate (PET) is one of the most common polymers used in packaging, construction, and agricultural industries [1,2]. Its semi-aromatic and semi-crystalline synthesis gives it high mechanical strength and barrier properties suitable for packaging. Many enzymes that decompose PET have been discovered since 2000, such as lipases and carboxyl ester hydrolases [3]. Both PET and MHET hydrolases attack the polymer ester bonds (Fig. 1) [4].

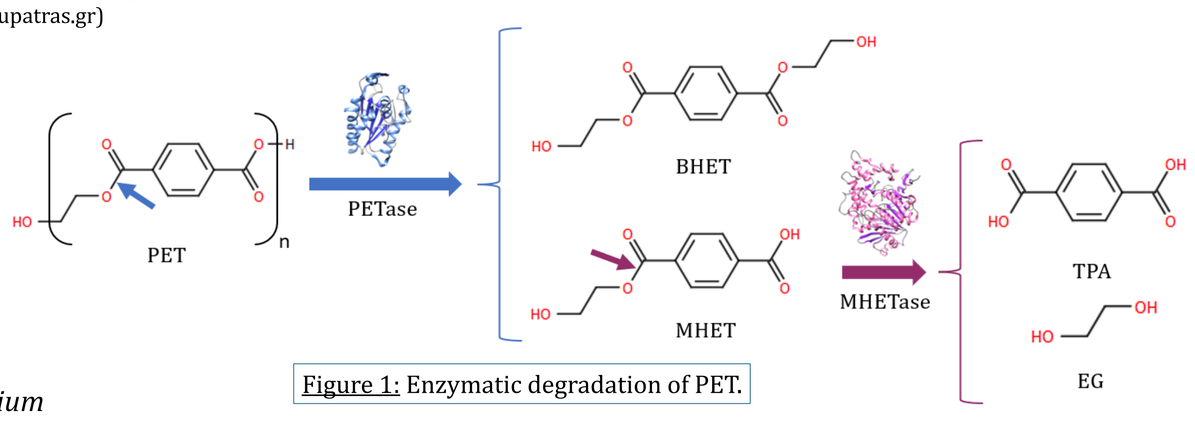

Figure 1: Enzymatic degradation of PET.

Objectives

This study focuses on investigation of structure-function relations of a ferulic acid esterase from *Fusarium oxysporum* (*Fo*FaeC) with degradation capacity against MHET [5]. Using structure-guided mutagenesis to mimick MHETase active site, an *Fo*FaeC variant, G122S, was created. The aim was to identify the structural determinants of MHETase activity by combining:

1. The determination of crystal structure of *Fo*FaeC variant, and
2. The Molecular Dynamics (MD) and Docking Simulations of both wild-type *Fo*FaeC and its variant.

X-ray Crystallography Experiments

Methodology: *Fo*FaeC variant was expressed in *Pichia pastoris* and purified using immobilized metal affinity chromatography. A mixture of 16mg/ml G122S and 5mM MHET, after 30min incubation on ice, was used for crystallization using sitting drop, vapor – diffusion method in the presence of already established crystallization condition [6,7].

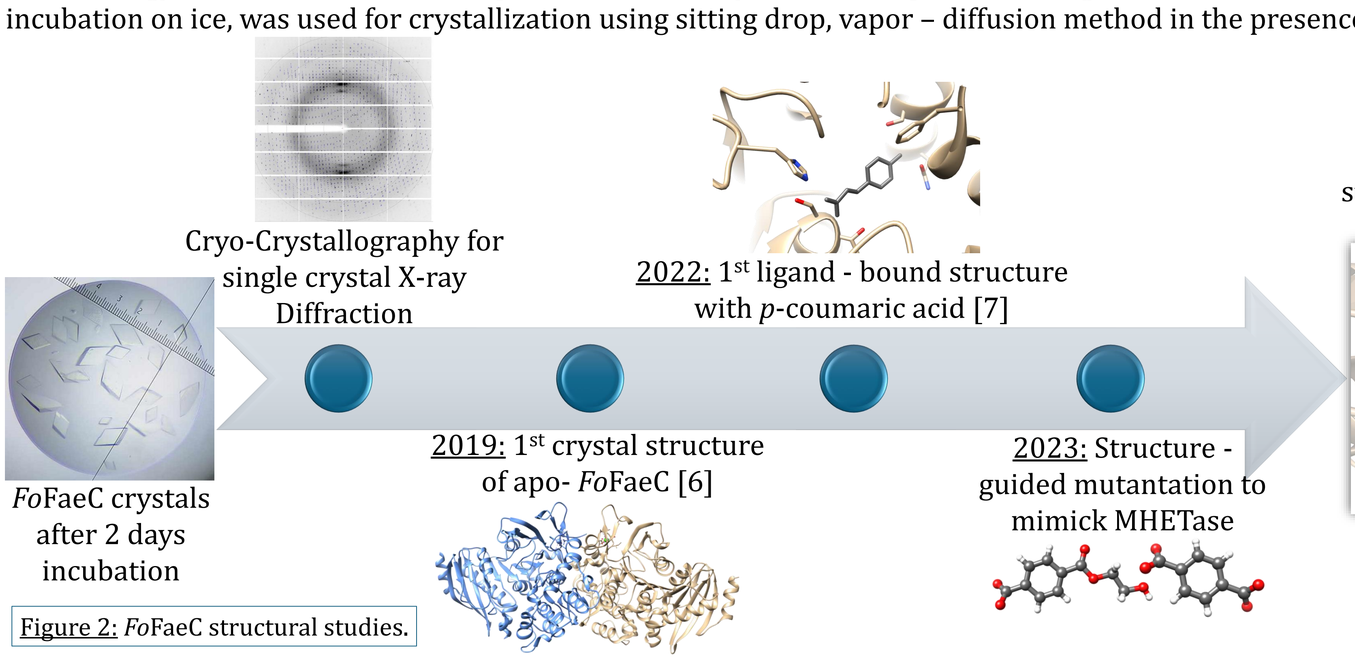

Figure 2: *Fo*FaeC structural studies.

Table 1: Diffraction data and refinement statistics for *Fo*FaeC_G122S structure. Values in parenthesis is for the outermost shell.

Data Collection		Multiplicity	3.4 (3.5)
Beamline	P13	No. of observations	624 449 (31 578)
Wavelength (Å)	0.9763	Unique reflections	181 348 (8991)
Space group	$P2_1$	R_{merge} (%)	0.078 (1.370)
Unit Cell Parameters		R_{pim} (%)	0.049 (0.851)
(a, b, c) (Å)	(68.0, 89.9, 115.1)	**Refinement Statistics**	
Resolution (Å)	89.93-1.58 (1.61-1.58)	R_{work} (%)	0.189
Completeness (%)	98.7 (99.0)	R_{free} (%)	0.230
Mean (I/SD(I))	6.1 (0.8)	RMSD, bond length (Å)	0.0090
$CC_{1/2}$	0.995 (0.532)	RMSD, bond angles (o)	1.792

Molecular Dynamics Experiments

Model coordinates: The initial coordinates → from *Fo*FaeC crystal structure (chain B, pdb: 6FAT) for the wild-type (WT) and from the crystallographic experiments for the mutant G122S (MT). Glycosylation was removed prior to the preparation of the systems. All resolved water molecules were retained in the structures. The model structures were embedded in a 12x12x12 nm^3 simulation box – cell and hydrated by around 55000 TIP3P water molecules [9]. The models contained one structural Ca^{2+} and 5 Cl^- anions to neutralize the system (~173k atoms in total). The Charmm36 Force Field was employed for the description of the polypeptide chains and ions [10]. MD simulations were performed in GROMACS v 2022.5 MD engine.
Model equilibration: Based on published protocols, all models were relaxed and equilibrated with gradual removal of constraints on the protein backbone-heavy atoms [11]. This totals in a production **sampling time** of 2 systems (WT, MT) x 4 (independent trajectories)/ system x 0.5μs = **4μs**.

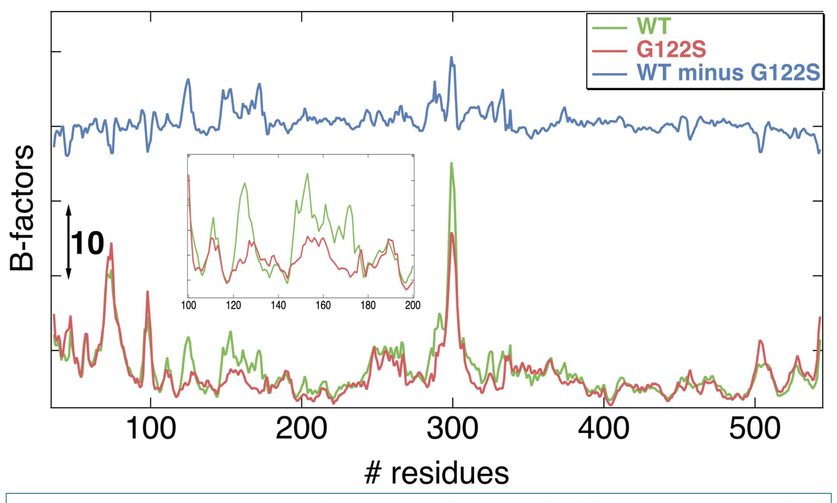

Diagram 1: Comparison between B-factors of wild-type *Fo*FaeC and G122S after 0.5μs MD simulation. B-factors have been calculated from RMSF through the equation $B = 8\pi^2(RMSF)^{2/3}$

For the analysis: PCA (Principal Component Analysis) and k-means clustering was performed by in-house scripts based on the sklearn python library. The Root-Mean-Square Fluctuations (RMSF) were calculated for the protein backbone atoms and weight-averaged based on the eigenvalues of the three main PCA components per replica that represent 35-40% of the total motion sampled per replicate trajectory. Four clusters were identified per system (WT, MT) by the k-means algorithm.

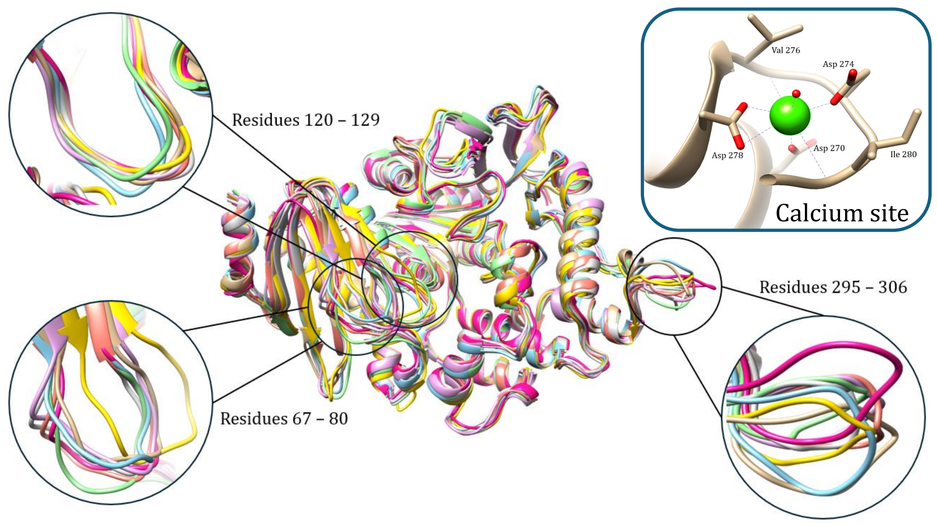

Figure 3: Alignment of 8 clusters (4 WT, 4MT) with emphasis on domains with greatest mobility.

Docking with MHET

Docking analysis was performed using the YASARA 21.6.17 software. Key conformation for *Fo*FaeC enzyme (wild-type and G122S) were obtained from the MD analysis and were used as initial coordinates for docking. The ligand was downloaded from the PDB (PBD code: 6QGA). The structure superimposed using MUSTANG and the resulting structure was used to perform local docking in YASARA.

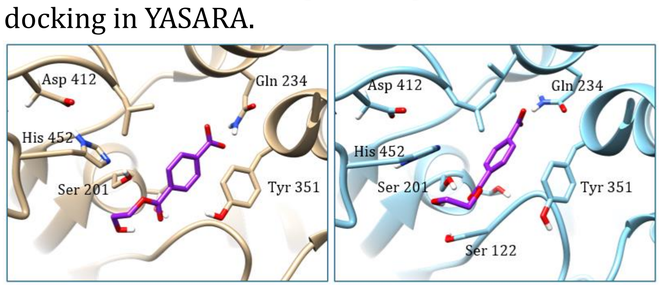

Figure 4: Comparison of MHET binding of WT and MT structure.

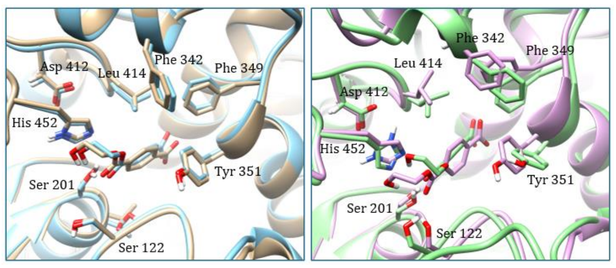

Figure 5: Different side chain conformation of Ser122 influencing binding of MHET.

Table 2: Average binding energy and standard deviation of WT and MT simulation (More positive values indicates stronger binding).

WT structures with MHET	
Average Binding Energy (kcal/mol)	5.068
Standard Deviation	0.103
MT structures with MHET	
Average Binding Energy (kcal/mol)	5.153
Standard Deviation	0.043

Conclusions

- First crystal structure of *Fo*FaeC variant (G122S) in complex with benzoic acid.
- Reduced mobility in the region around residues 100 – 200 due to the mutation G122S leads to increased *Fo*FaeC activity against MHET.
- Docking confirms the enhanced binding of MHET in the active site of G122S variant.
- The decreased standard deviation of binding energies of MHET at the catalytic center of MT structures indicates an energetically more stable conformation.

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