Structural studies and molecular dynamics simulations of a ferulic acid esterase variant active on PET oligomers

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Introduction

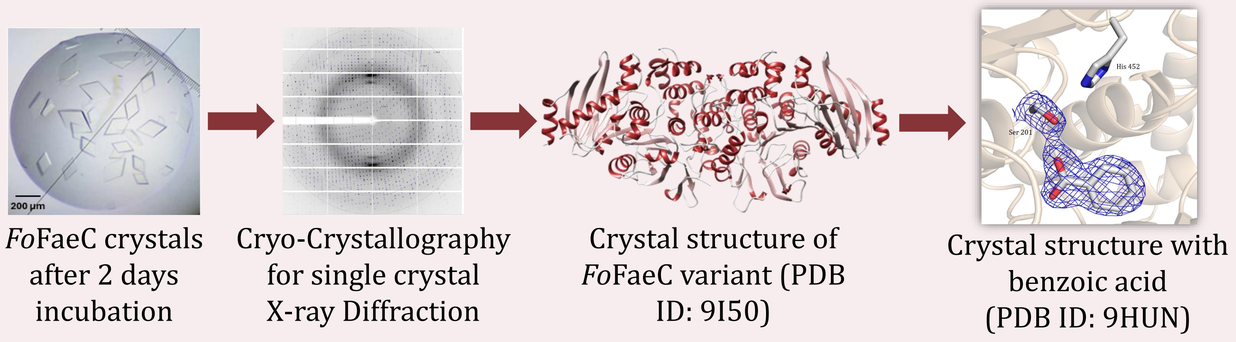
This study focuses on the investigation of the structure-function relationship of a ferulic acid esterase from *Fusarium oxysporum* (*Fo*FaeC) with degradation capacity against MHET [1]. Using structure-guided mutagenesis to mimick MHETase active site, an *Fo*FaeC variant, G122S, was created. The aim was to identify the structural determinants of MHETase activity by combining:

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

Methodology

X-ray Crystallography

*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 [2,3].



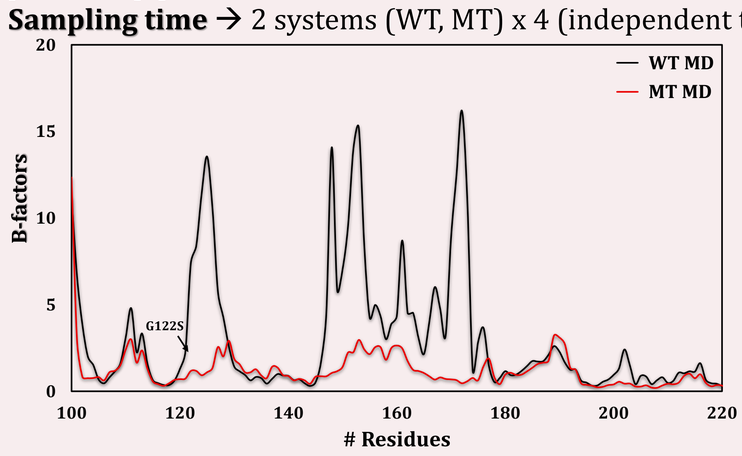
*Fo*FaeC crystals after 2 days incubation | Cryo-Crystallography for single crystal X-ray Diffraction | Crystal structure of *Fo*FaeC variant (PDB ID: 9I50) | Crystal structure with benzoic acid (PDB ID: 9HUN)

Figure 1: Structural characterization methodology.

Molecular Dynamics Simulations

MD simulations were performed in GROMACS v 2022.5 MD engine described by CHARMM36 potential [4].

Sampling time → 2 systems (WT, MT) x 4 (independent trajectories)/ system x 0.5μs = **4μs.**



Docking with MHET

Docking analysis was performed using the YASARA 21.6.17 software. MD simulations yield structures with the best binding energy and the lowest dissociation constant for WT and MT.

Diagram 1: Comparison between B-factors of the apo structures of wild-type *Fo*FaeC and G122S focusing on the region of mutation along 0.5μs MD simulation.

Results

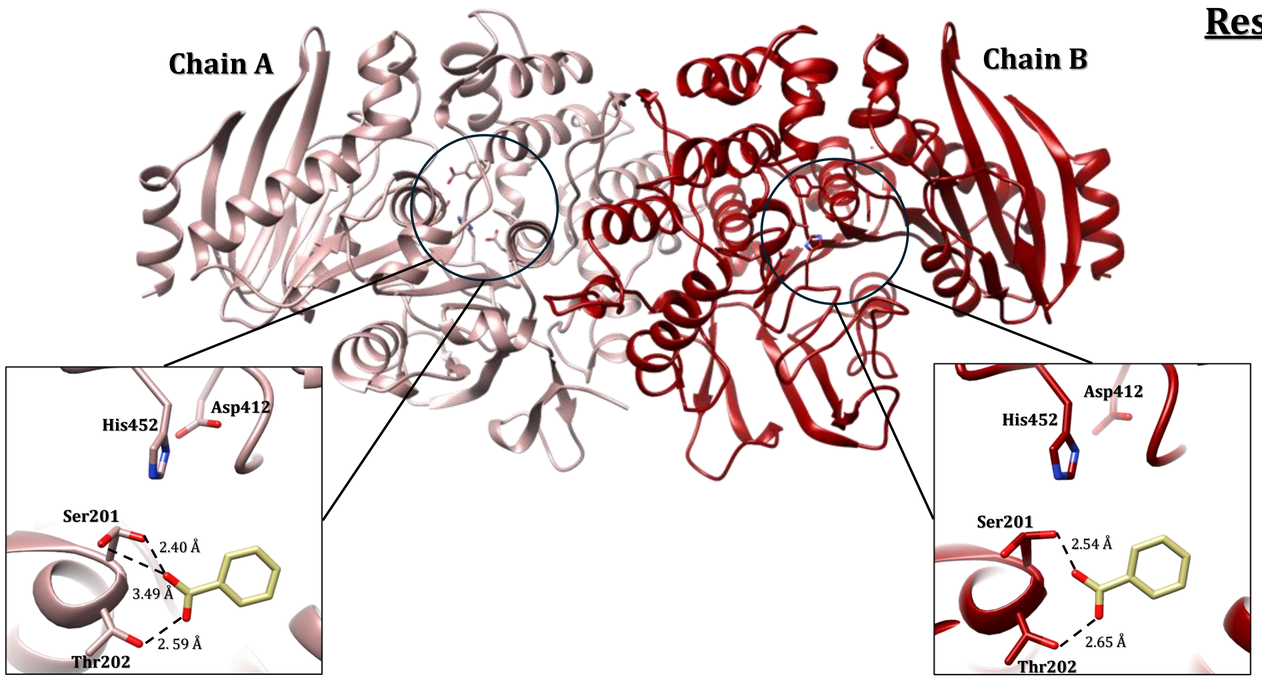


Figure 2: Cartoon representation of the dimeric form of *Fo*FaeC variant in complex with benzoic acid.

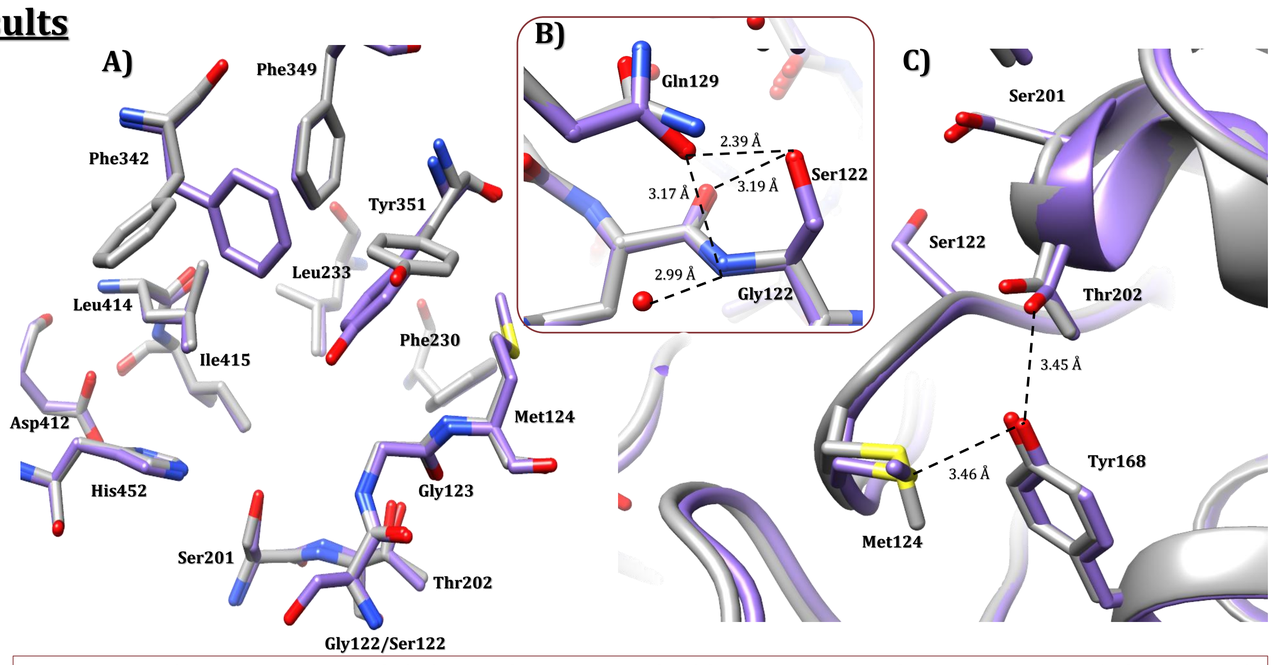


Figure 3: Stick representation of A) the catalytic center of wild-type *Fo*FaeC and *Fo*FaeC variant, B) the mutated region and C) the region with reduced B-factors (gray color: wild-type *Fo*FaeC, purple color: *Fo*FaeC variant).

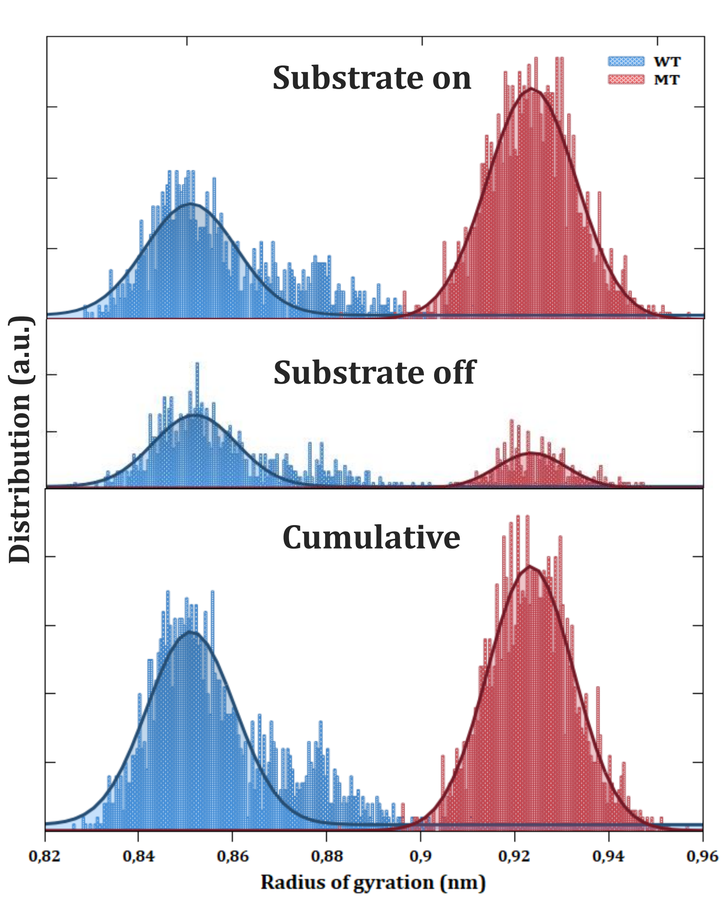


Diagram 2: Radius of gyration around the MHET formed by the main chain atoms of Ser201, His452, Asp412, Gly122/Ser122, Gly123, Met124, Thr202, Phe230, Leu233, Gln234, Ser237, Phe342, Phe349, Tyr351, Leu414, Ile415 across 0.5μs MD simulation.

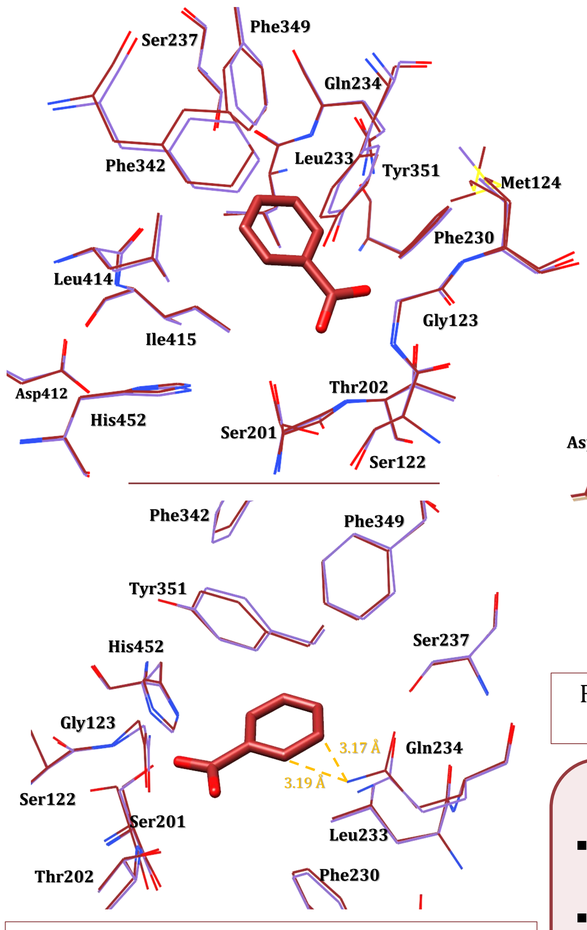


Figure 5: Stick representation of the substrate binding pocket of *Fo*FaeC variant in complex with benzoic acid (red color) superimposed on the apo structure of *Fo*FaeC variant (purple color).

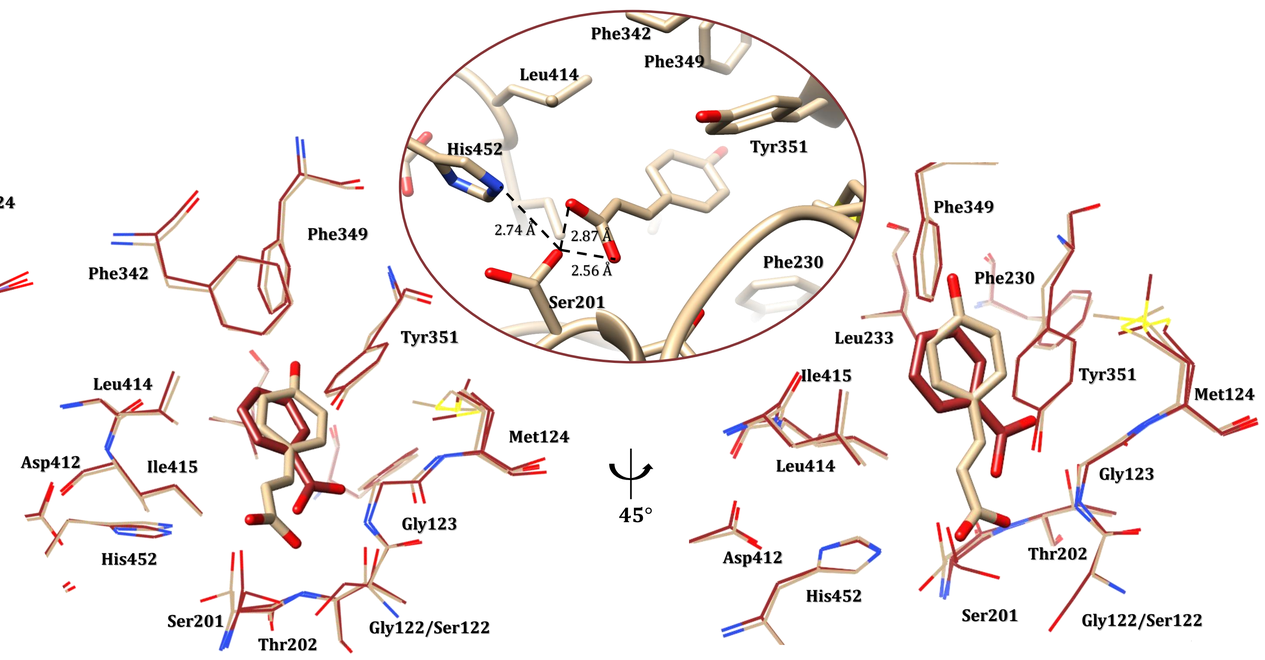


Figure 4: Stick representation of the substrate binding pocket of *Fo*FaeC variant in complex with benzoic acid (red color) superimposed on the wild-type *Fo*FaeC in complex with *p*-coumaric acid (tan color).

Conclusions – Future work

- Crystal structures of *Fo*FaeC variant (G122S) and *Fo*FaeC variant in complex with benzoic acid (PDB IDs: 9I50, 9HUN).
- Ser122 side chain forms hydrogen bond with Gln129 and a solvent molecule → stabilizes the region of mutation.
- G122S variant with increased activity against MHET shows reduced mobility in the region around residues 100 – 200 (Diagram 1 from MD simulations).
- The radius of gyration of structures of the wild-type enzyme are about 1 Å smaller than those of mutant G122S.
- MD simulations of structures with docked substrates, where the ester bond is at the minimum distance to the catalytic residues are underway.

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