

# Biochemical characterization and NMR study of a PET-hydrolyzing cutinase from *Fusarium solani* *psi*

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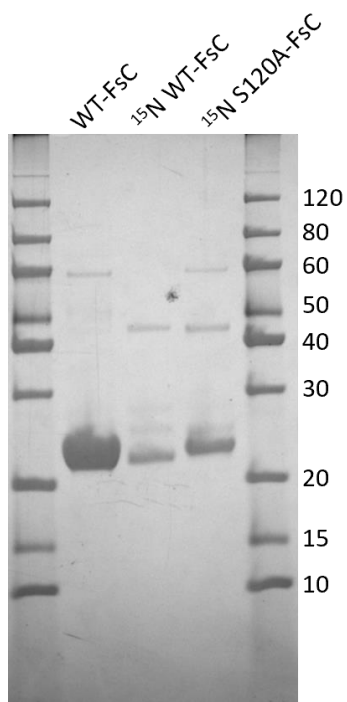
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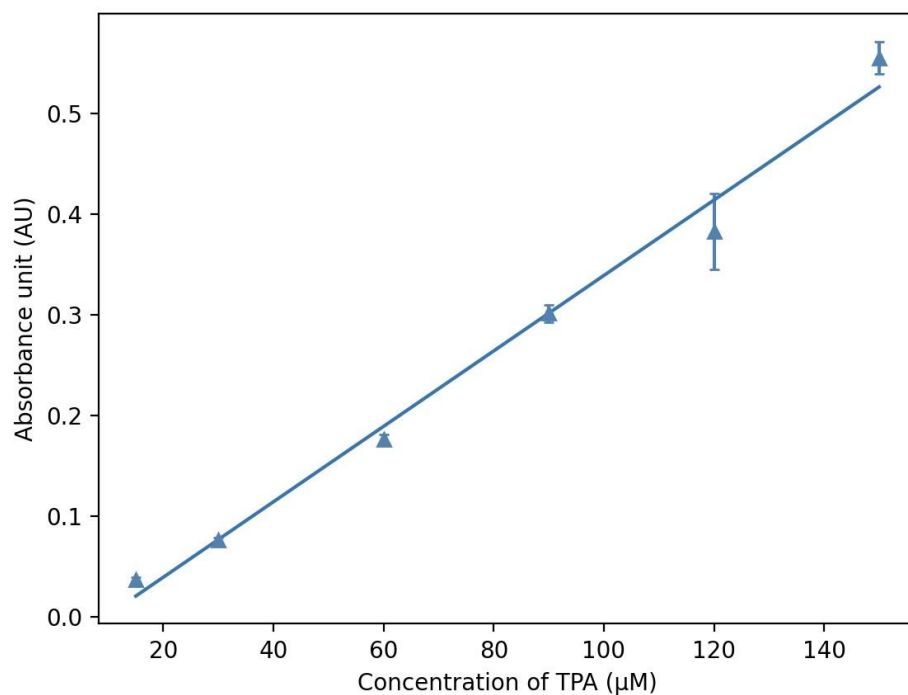
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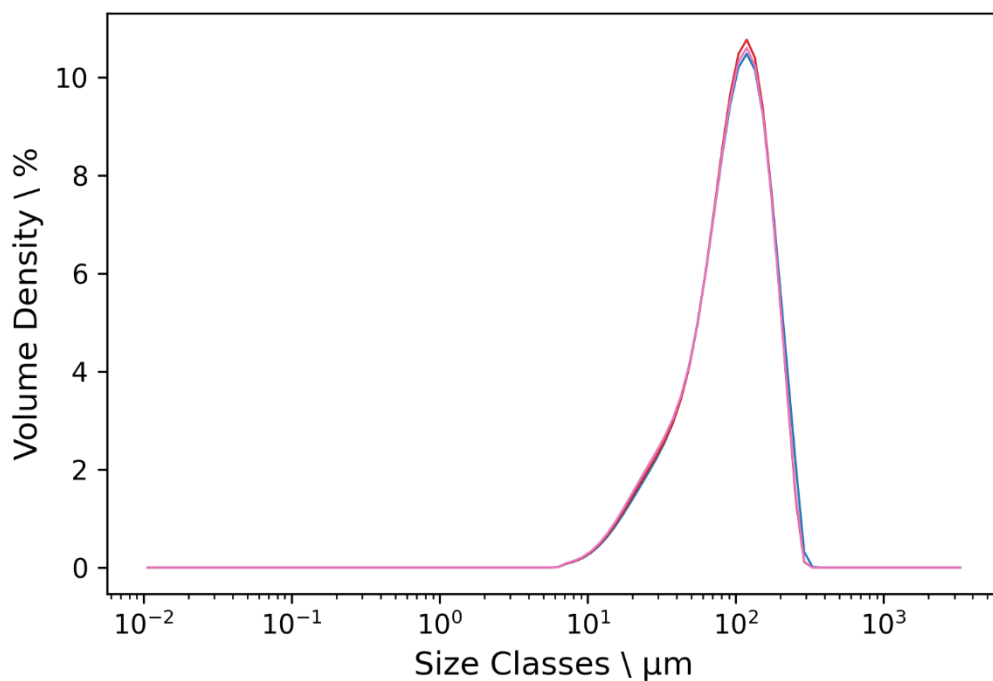
**SUPPLEMENTARY INFORMATION**



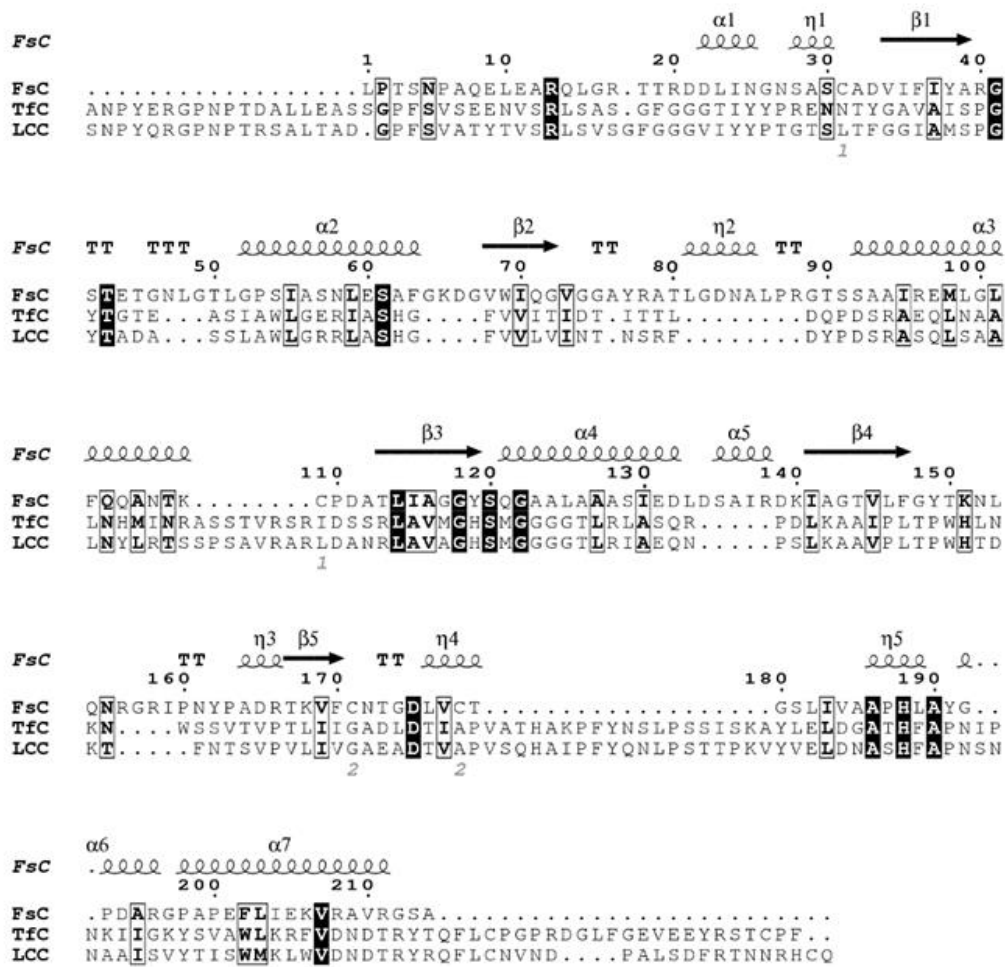
**Figure S1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).** The picture of the stained gel shows the FsC samples used for activity assays (WT-FsC) and NMR titration experiments (<sup>15</sup>N S120A-FsC), as well as a sample of <sup>15</sup>N WT-FsC. The molecular weights (in kDa) of the standards (PAGE-MASTER Protein Standard Plus from GenScript) are indicated for the bands in right-most standard lane.



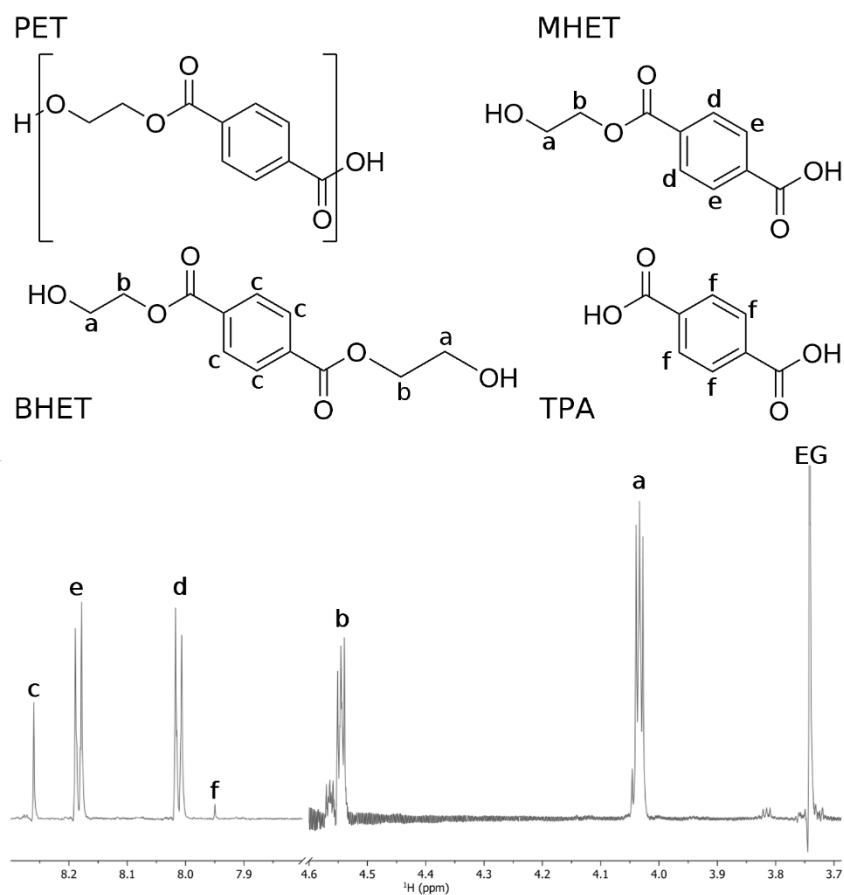
**Figure S2. Terephthalic acid (TPA) standard curve.** Measured absorbance values at 240 nm as a function of TPA concentration. Linear regression was performed and gave a linear function of  $A_{240} = 0.0038 \cdot [\text{TPA}] - 0.0359$ , with  $R^2 = 0.99$ .



**Figure S3. Analysis of particle size distribution of PET powder.** The figure shows on overall of five curves describing the distribution of weight classes in a sample of crystalline PET powder (GoodFellow product code ES306031) dispersed in 96% ethanol. The following particle size parameters were determined. Volume-weighted mean diameter,  $D[4,3] = 103 \pm 1 \mu\text{m}$ ; surface area-weighted mean diameter,  $D[3,2] = 65.3 \pm 0.7 \mu\text{m}$  and specific surface area =  $92 \pm 1 \text{ mm}^2 \text{ mg}^{-1}$ .



**Figure S4. Multiple-sequence alignment (MSA) of three cutinases.** The MSA was performed in the Expresso/T-coffee server<sup>1</sup> and the visual representation was made using the ESPrpt 3.0 server<sup>2</sup>. FsC (*Fusarium solani pisi* cutinase; PDB 1CEX), TfC (*Thermobifida fusca* cutinase; PDB 5ZOA), LCC (leaf-branch compost cutinase; PDB 4EB0). Secondary structure elements from FsC are shown on top (helices with squiggles, β-strands with arrows and turns with TT letters). The bottom numbers (1 and 2) represent the disulphide bonds in FsC. α and η represent alpha- and 3<sub>10</sub>-helices, respectively. Identical and similar residues are highlighted black and boxed white, respectively.



**Figure S5. Chemical structures and chemical shifts.** The structures of polyethylene terephthalate (PET), bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalic acid (MHET), and terephthalic acid (TPA) are shown. The chemical shifts corresponding to  $^1\text{H}$  and  $^{13}\text{C}$  resonances (in ppm) were assigned at pD 6.5 and 313 K as **a**: 4.02, 59.8; **b**: 4.53, 63.8; **c**: 8.19, 129.5; **d**: 8.01, 128.8; **e**: 8.18, 129.5; **f**: 7.94, 129.5. The  $^1\text{H}$  NMR spectrum under the structures shows an overview of the assigned resonances, as well as the free ethylene glycol (EG) peak at 3.74 ppm.

## REFERENCES

- (1) Armougom, F., Moretti, S., Poirot, O., Audic, S., Dumas, P., Schaeli, B., Keduas, V., and Notredame, C. (2006) Espresso: automatic incorporation of structural information in multiple sequence alignments using 3D-Coffee. *Nucleic Acids Res.* 34, W604-608.
- (2) Robert, X., and Gouet, P. (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* 42, W320–W324.