

# BIOTECHNOLOGY for A CIRCULAR BIOECONOMY

28 - 29 March 2023 | AFOB-EFB Virtual Conference

## Analysis of PET degrading enzymes by bioinformatic tools

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### Abstract

PET hydrolases are enzymes that have been shown to act upon PET as a substrate. These enzymes usually adopt an  $\alpha/\beta$  hydrolase fold and are from the classes of esterases, lipases, cutinases, and hydrolases.

Here, we have done sequence alignment by ClustalW of the sequences corresponding to the entries available in the PAZy database ([pazy.eu](http://pazy.eu)) with the addition of a highly efficient *I. sakaiensis* PETase mutant W159H/S238F and analyzed the results. The aligned sequences included several different well-aligned segments, which were as follows: 18 single-amino acid segments, 13 two-amino acid segments, 10 three-amino-acid segments, 1 four-amino acid segment, 1 six-amino acid segment and 1 eight-amino acid segment. Additionally, at position 238, which is adjacent to a highly conserved His237, the most common amino acids were F, T, S, Y, W, L and G, whereas at position 159, the most common amino acids were W, H, I and L, flanked by a conserved three- and eight-amino acid region. These positions seem to be critical for the improvement of the PET hydrolytic activity based on the comparison of *I. sakaiensis* PETase mutant W159H/S238F and wt enzyme.

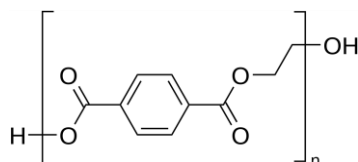
Using AlphaFold 2.0 we have predicted the structures of all enzymes available in the database whose structures haven't been previously reported and the presence of the  $\alpha/\beta$  hydrolase motif has been observed. The sequences were also analyzed by SIAS ([imed.med.ucm.es](http://imed.med.ucm.es)).

### Acknowledgement

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### Introduction

Polyethylene-terephthalate (PET) is a cheap to produce durable plastic with many desirable properties. Because of this, its use has become widespread. Because it can't be easily and efficiently recycled and upcycled, accumulating plastic waste is becoming a global problem.





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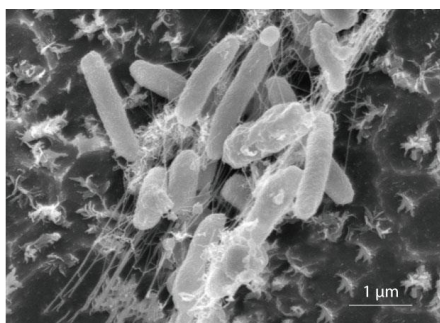
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*Ideonella sakaiensis* is a bacterium identified from an environmental sample in Japan. It has been shown to degrade PET at room temperature, unlike other PET hydrolases known at the time. The enzyme responsible for this action was identified and named *Is* PETase (Yoshida et al., 2016).



### Materials and methods

#### Database

A database containing reported plastic-degrading enzymes has been developed by Bucholz et al (Buchholz et al., 2022, [pazy.eu](https://pazy.eu)). The sequences of the proteins in the database were scraped from the NCBI ([ncbi.nlm.nih.gov](https://ncbi.nlm.nih.gov)) and UniProt ([uniprot.org](https://uniprot.org)) online databases. The sequences were truncated by removing signal peptides detected by SignalP 6.0 ([services.healthtech.dtu.dk](https://services.healthtech.dtu.dk)).

#### Alignment

The described sequences were aligned using ClustalW ([genome.jp](https://genome.jp)) with default parameters and visualized by ESPript 3.0 ([esprict.ibcp.fr](https://esprict.ibcp.fr)).

#### Sequence identity and similarity

Sequence identity and similarity were visualized using the SIAS server ([imed.med.ucm.es](https://imed.med.ucm.es)). The obtained results were displayed in a table and colored according to their values.

#### Structural modelling

Sequences were modelled using AlphaFold 2.0 ([online collaborative version](https://online.collaborative.version)). The obtained structures were visually analyzed for the presence of the  $\alpha/\beta$  hydrolase structural motif characteristic of most esterases.

The generated models were compared by pairwise structure alignment on [pdb](https://www.rcsb.org) ([www.rcsb.org](https://www.rcsb.org)).

### Results

At position 238, adjacent to a highly conserved His237, the most common amino acids were F, T, S, Y, W, L and G, whereas at position 159, the most common amino acids were W, H, I and L, flanked by a conserved three- and eight-amino acid region. These positions seem to be critical for the improvement of the PET hydrolytic activity based on the comparison of *Is* PETase mutant W159H/S238F and wt enzyme.

	β7	TT	α5	β8	TT	T
	180	190	200	210		
ISPETase	TLIFACENDSIA	AV.NSSALP	YDMSR.NAK	CFLEINGGSH	SCANS	.....
IsPETase_W159H_S238F	TLIFACENDSIA	AV.NSSALP	YDMSR.NAK	CFLEINGGSH	SCANS	.....
RgpETase	TLIVSCENDSIA	PN.SSHGFP	YNNQMR.NRKA	LILINGGSH	SCANS	.....
PET12	TLVVGQGDIT	AAP.AEWAIP	YNNRMSAST	GKALEVRGGS	SHFCVTSST	.....
Enzyme_406	TMIIACQADV	VAV.SVHASR	YNNQPGT	TRAYFELALG	SHSCANTGYP	.....
PET6	TLIFACQDAV	VAV.AQHASR	YNNQPGT	TRAYFELALG	SHSCANTGYP	.....
Enzyme_403	TLVIACENDV	VAV.NSHASR	YNNRPSST	DKALEINGGSH	SCANDGGS	.....
PET5	TLVIACENDV	VAV.NSHASR	YNNRPSST	DKALEINGGSH	SCANDGGS	.....
PLB628	TLIFACESDVI	AV.GSHADP	YEAIPD	STDKAFELNNGS	RYCCNG	.....
PLB629	TLIFACESDVI	AV.GSHADP	YEAIPD	STDKAFELDGGG	HICANGSSGF	.....
PpCuiA	TLIFACQDAV	VAV.GSHASR	YNNRPSST	DKALEINGGSH	SCANGSS	.....
PE-H	TLIFACESDVI	AV.LQHASR	YNNRPSST	DKAFVEINGGSH	RYCCNGG	.....
PpelaLip	TLIFACESDVI	AV.GSHASR	YNNRPSST	DKAFVEIDGGN	HICANGAAS	.....
esterase_Mg8	TLIFACQDAV	VAV.AQHASR	YNNRPSST	DKALEINGGSH	SCANGSS	.....
PET2	TLIFACQDAV	VAV.AQHASR	YNNRPSST	DKAFVEINGGSH	SCANGSS	.....
Enzyme_504	TMIFGQDDIT	AV.FSHAI	YNNRPSST	DKAFVEIDGGN	HICANGSS	.....
Thc_Cut1	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
Tha_Cut1	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
TfCut_2	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
Cut2	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
FTA-1	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
WSH03-11_Tfu_0883	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
TfH42_Cut1	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
Enzyme_702	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
Enzyme_701	TLIGADLDI	AV.ATHAKP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....
WSH03-11_Tfu_0882	TLIGADLDI	AV.LTHARP	YNNRPSST	SKALELDGAT	HFAFNIPNK	.....

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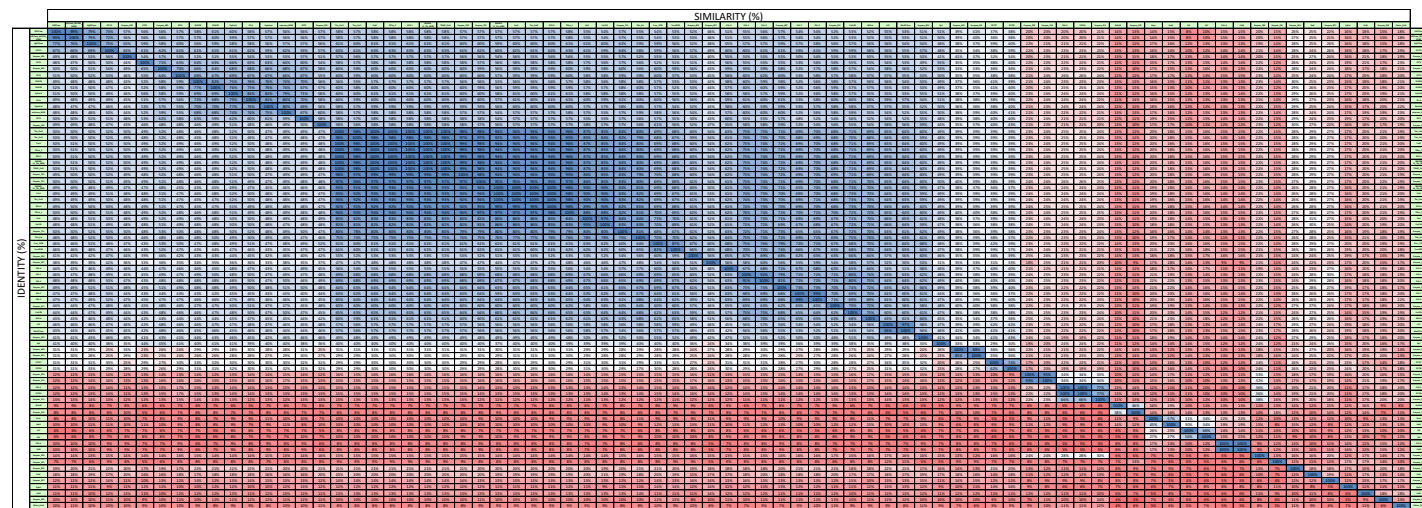
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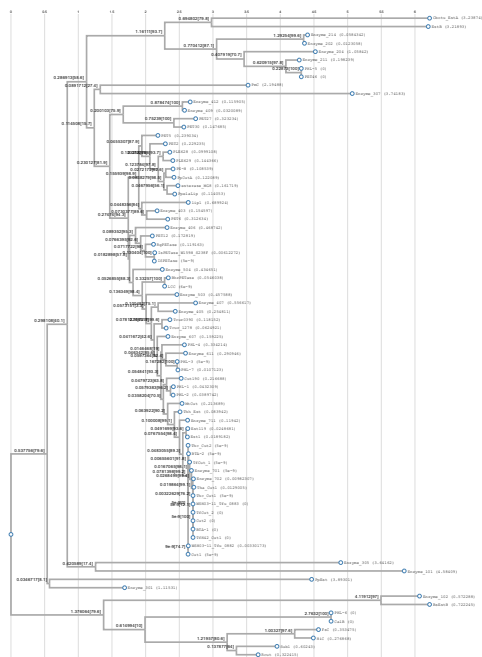
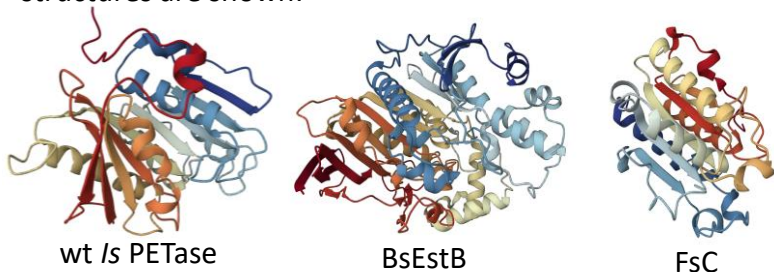
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SIAS analysis correlated well with the phylogenetic tree generated from the ClustalW aln file. All structures modelled by AlphaFold 2.0 included the  $\alpha/\beta$  hydrolase motif, where smaller similarity values between the analyzed sequences visually seem to affect other structural aspects of the enzymes more than the  $\alpha/\beta$  hydrolase motif. This probably correlates with different stabilities of the well-studied enzymes described in the literature, as well as other catalytic aspects. The modelled *Is* PETase and some other structures are shown:



### References

1. Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., & Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, 351(6278), 1196–1199. <https://doi.org/10.1126/science.aad6359>
2. Buchholz, P. C., Feuerriegel, G., Zhang, H., Perez-Garcia, P., Nover, L. L., Chow, J., Streit, W. R., & Pleiss, J. (2022). Plastics degradation by hydrolytic enzymes: The plastics-active enzymes database— pazy. *Proteins: Structure, Function, and Bioinformatics*, 90(7), 1443–1456. <https://doi.org/10.1002/prot.26325>