Analysis of PET degrading enzymes by bioinfomatic 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 α/β 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) 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 α/β hydrolase motif has been observed. The sequences were also analyzed by SIAS (imed.med.ucm.es).

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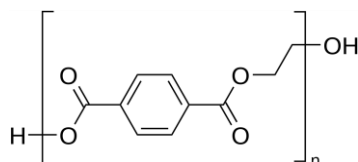
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Ideonella sakaiensis is a bacterium identified from an environmental sample in Japan. It has been shown to degrade PET at room temperature, unlike other PET hydrolases known at the time. The enzyme responsible for this action was identified and named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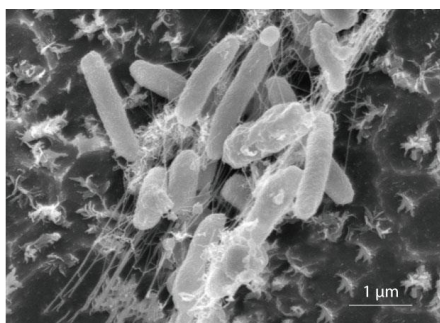
Materials and methods

Database

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

Alignment

The described sequences were aligned using ClustalW (genome.jp) with default parameters and visualized by ESPript 3.0 (espript.ibcp.fr).

Sequence identity and similarity

Sequence identity and similarity were visualized using the SIAS server (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). The obtained structures were visually analyzed for the presence of the α/β hydrolase structural motif characteristic of most esterases.

The generated models were compared by pairwise structure alignment on pdb (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.

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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 α/β hydrolase motif, where smaller similarity values between the analyzed sequences visually seem to affect other structural aspects of the enzymes more than the α/β 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:

wt *Is* PETase   BsEstB   FsC
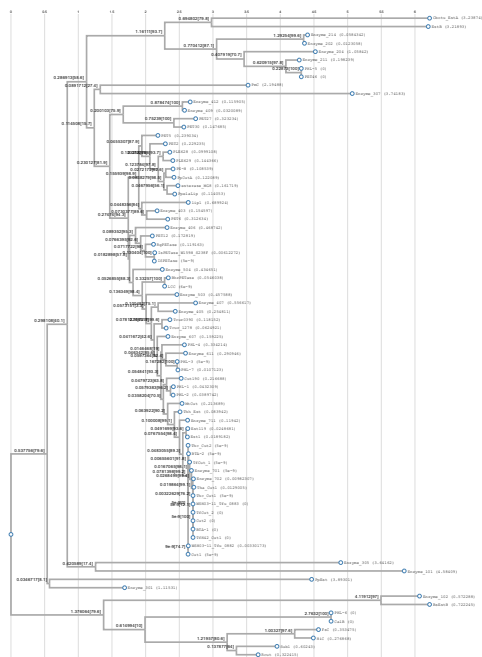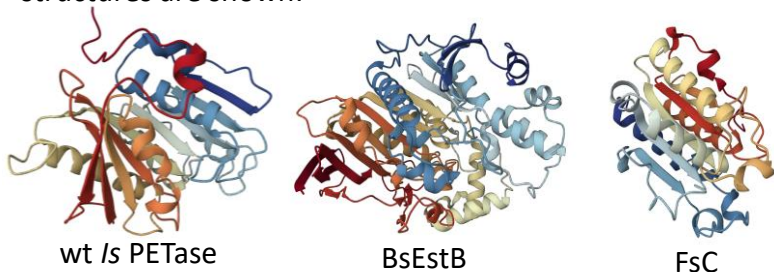
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