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CHARACTERIZATION OF PET HYDROLASES ASSOCIATED WITH MICROPLASTIC DEGRADING BACTERIA: AN *IN-SILICO* ANALYSIS

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Abstract

Development of PETase variants with efficient potential of polyethylene terephthalate (PET) degradation is a sustainable route to microplastic (MP) bioremediation. Current study was initiated to explore the characteristics of PETases which could be helpful in enzyme modification via genetic engineering. For this purpose, three isoforms of enzyme associated with three different bacteria Piscinibacter sakaiensis, Streptomyces labedae and Compostimonas suwonensis were selected. Amino acid sequence was retrieved from UNIPROT database and subjected to analysis via SOPMA tool, SWISS-MODEL server and MEME suite. The alpha helix, extended sheet and random coil observed were ranged between 78-113, 41-204 and 149-729, respectively. Monomeric three-dimensional (3D) configuration was observed in A0A0K8P6T7 and A0A2M9BCQ1 and dimeric configuration in A0ABP6QVC8. Conserved motifs (n = 11) were predicted for each of the three isoforms of PETases. These conserved domains might be targeted for engineering in order to enhance their binding affinity for PET. Considering the properties of PETases unraveled in current project and implementation of rational protein engineering alongwith in-silico strategies, might be helpful for the development of PETase super enzymes.

1. Introduction

PETases belong to esterase enzymes, which transform polyethylene terephthalate (PET) via hydrolysis to monomers of mono-2-hydroxyethyl terephthalate (MHET) (Kawai et al. 2019). At present, PETases got considerable attention due to their role in polyethylene terephthalate (PET) microplastic degradation. PET pollution has been increasing significantly due to extensive production of single-use plastics which is playing havoc with health of human and marine life (Lopez-Lorenzo et al. 2024; Pilapitiya and Ratnayake 2024; Khaele et al. 2023). Conventional methods are not able to successfully remediate the PET waste due to being time consuming, greater exposure to plastic hazards for workers, incomplete degradation and high cost (Kang et al. 2020).

Literature documents wide variety of bacteria exhibiting potential of PET degradation via PETases. i. e., *Pseudomonas fluorescens*, *Acinetobacter baumannii*, *Staphylococcus cohnii*, *Bacillus mycoides*, *S. xylosus*, *B. cereus*, *B. pumilus*, *B. thuringiensis*, *B. amyloliquefaciens*, *B. sphericus*, *Delftia comamonas*, *Stenotrophomonas*, *Paenibacillus macerans*, *Rahnella aquatalis*, *Micrococcus luteus*, *M. lylae*, *Rhodococcus ruber*, *Arthrobacter viscosus*, *P. aeruginosa*, *P. citronellolis*, *B. flexus*, *Kocuria palustris*, *B. subtilis*, *Ideonella sakaiensis*, *Saccharomonospora viridis* and *B. gottheilii* (Auta et al. 2017; Giacomucci et al. 2019; Harshvardhan and Jha 2013; Jeon and Kim 2015; Kawai et al. 2014; Nowak et al. 2011; Oberbeckmann et al. 2014; Peixoto et al. 2017; Sudhakar et al. 2007; Wrobel et al. 2024; Yoshida et al. 2016).

As per previous reports, rate of PET hydrolysis is a result of interaction between PET and PET hydrolases (Kawai et al. 2019). In a reported study, hydrolysis rate of enzyme was enhanced through optimization of active site via insertion of mutations which resulted in greater affinity of active site for PET polymer (Karunatillaka et al. 2020; Rathod and Biswas 2026). Additionally, two variants of PETase with optimum activity have been developed which exhibit thirty-eight times more hydrolytic potential as compared to wild type enzyme. These variants are DuraPETase and FAST-PETase (Barclay and Acharya 2023; Liu et al. 2023). Keeping in view these facts, we initiated current study in order to get insights into the detailed characteristics of PET hydrolases which might be consulted to produce laboratory manipulated super enzymes.

2. Methodology

2.1 UNIPROT database

To get the sequences of PETases, we consulted UNIPROT database (Available at; https://www.uniprot.org, accessed on May 2025). PETases from three microplastic degrading bacteria including *Piscinibacter sakaiensis*, *Streptomyces labedae* and *Compostimonas suwonensis* were selected. Sequences, length, accession numbers and associated bacterium details of PETases are mentioned in Table 1.

Table 1: ***Accession numbers, bacteria, length and sequences of PET hydrolases retrieved from UNIPROT database, documented in current study***

#	Acc. No.	Bacterium	Length	Sequence
1	A0A0K8P6T7	*Piscinibacter sakaiensis*	290	MNFPRASRLMQAAVLGGLMAVSAAATAQTN PYARGPNPTAASLEASAGPFTVRSFTVSRPSGY GAGTVYYPTNAGGTVGAIAIVPGYTARQSSIK WWGPRLASHGFVVITIDTNSTLDQPSSRSSQQ MAALRQVASLNGTSSSPIYGKVDTARMGVMG WSMGGGGSLISAANNPSLKAAAPQAPWDSST NFSSVTVPTLIFACENDSIAPVNSSALPIYDSMS RNAKQFLEINGGSHSCANSGNSNQALIGKKG VAWMKRFMDNDTRYSTFACENPNSTRVSDFR TANCS
				MAMNRRTFLGLGAAGAVAAVHAYGTTAATA SSLAPADAPTQRFAIGVREYAWSRGSRRLTTRI FYPATGTPGGNPVVNAPIADGVFPICEFSHGL GGNPESYAPLVRPLAEAGFVVPAPVFPTTSSGT QGNVQDVYNGNQSMDVSQVITNTLALNTTA GDPFAGHLDTTTGVGVAGHSLGGMTTHGLL

2	A0ABP6QVC8	*Streptomyces labedae*	443	TAWPDSRIAAAVPFACVDMGNPSSTVAAKVLF VHGDQDTLTQYSSARQAYGELPAPKAFLTHR GTGHDQYIWSGYNHTQTVATYVDWMRWSL YADTAARDRLPADATSNTSTTTWEAVLTPLPT GPIVSLRSRANNQYVCAENAGTDPLIANRTAI GDWERFDLIDLGGGAVALRARVNGRYVCAE NAGANALIANRTAVGPWETFQRVNNSDGTTS LRAQANNLYVSAGNAGTAPLIANRTTIGPSESF DLING
3	A0A2M9BCQ1	*Compostimonas suwonensis*	1046	MPKSVRHWKHIALATALCFAATPFLAGAPAYA ASSSDDSATVAQGDDWIVSRVAAGYQVSKTL AEPIEFRDAAPTLWADGVDLGIANQSLDGLTI TVTTDDPVVLTAGDVALGWEGEGDPTVETPV PDAGAGALGAQRLSKALVVDESSIASILADDP ATHGQYSVERDDYDLGDEAEDIRGFGRKGE MRAALFMPVGAAGERPVVLFLHGRHTSCSGG TRNPDSWPCNPDQVDVASYLGYNDAAEALAS RGYAVVSISANAINALDGTLSDDNGAVARAQ LILDHLELLREANEGPVDGLSPSLKGRLDLDH VGVMGHSRGGDGAVRAALLNAELEKPFGIEA VLPLAPTDFGRMTLPDVPTATILPYCDGDVSN QQGQHFFDDSQHAFDDNVLRSSVLVMGANH NFFNTSWTPSKYAFSASDDWNNNNGDPDCKY GVSPTRLTDDEQYDVGTAYVTAFFRLTMGDE QQFLPMFDGSDAKPVSAGRADVRVSASLPAST RYDVNNFDKPDTDVRVLGAGTYQYCASLSAR PVPGEQPYCVNSLTTSQVPDFTPANFAQSVPA TPSLHFTYTAPANANALAGEIRVPLDAGKFDV TSYEDLSFRVSPDETATGTTDLTVTLADGSGA TASVHASEYGDALTVLPGTVSPLRKVLLQQLTI PTGDFAGVNLSDIREVRFTAPRAAGGVLLGDL AFLATPSVGDAKVSTRPVLSIPDVNIEEGDGPA TAYVPVLLSRSQDVPTTAWFSAVGTANGKVD LALKKLTFEPGQTCIAVPVPTKGDNEQGTTQS AKFKMTVSNTQEGSTIGDSFGYLTVREDDGV VTPTIPSVPPRGGNPGTPEIPGGDPLPSAPAVG VQGDACAEATAEPGTLTVSPASAERGDSVTIT GSGFRSGESVSFAFHSDPVDLGSLVSTDGTVSL TTTVPDDAEFGAHEFQALGAGSAFTATGALE VVDPNAPTDEPTDTPTDVPTDTPTDVPTDTPS DTPTDAPLDPGSDGTTPGSTTSGTAAGLASTG FEGAGWALLALLLIGGGATVFFARRKRVSQD DAS

2.2 SOPMA tool

SOPMA Protein Secondary Structure Prediction tool available on server of NPS@ (Network Protein Sequence Analysis) (Available at; https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html, accessed on May 2025) was employed to analyze the secondary structure (2D) attributes (α-helix, extended sheet and random coil) of PETases. Protein sequence input was given as FASTA format with output width of 70. Values of 17 and 8 were chosen for window width and similarity threshold, respectively.

2.3 SWISS-MODEL server

SWISS-MODEL, a comparative modelling computational server was consulted via ExPaSy web server (https://swissmodel.expasy.org, accessed on May 2026)to interpret the three-dimensional (3D) configuration of PETases. This server provided us tertiary structures along with Ramachandran plot analysis.

2.4 MEME suite

Motif based sequence analysis tool, The MEME Suite 5.5.9 (Available at; https://meme-suite.org/meme/, accessed on May 2025) was employed to predict the conserved domains of PETases. Classic motif discovery mode and any number of repetitions (anr) was selected as site distribution. FASTA sequence was given as input data via type-in mode.

3. Results

3.1 Prediction of secondary (2D) configuration of PETases

The 2D configuration was determined at three levels. i. e., alpha helix, extended sheet and random coil. Highest number of amino acids (113) were found as part of alpha helix in enzyme A0A2M9BCQ1 followed by 100 in A0A0K8P6T7 and then 78 in A0ABP6QVC8. Two hundred and four amino acids constituted the extended sheet of A0A2M9BCQ1 followed by seventy-five and forty-one for A0ABP6QVC8 and A0A0K8P6T7, respectively. Highest proportion of amino acids (729) taking part in random coil formation was observed in A0A2M9BCQ1 followed by 290 in A0ABP6QVC8 and 149 in A0A0K8P6T7 (Table 2 and Figure 1).

Table 2: ***Predicted of secondary (2D) configuration of PETases documented in current study, using SOPMA tool***

Enzyme	Alpha helix	Extended sheet	Random coil
	Number of amino acids (% age)		
A0A0K8P6T7	100 (34.48)	41 (14.14)	149 (51.38)
A0ABP6QVC8	78 (17.61)	75 (16.93)	290 (65.46)
A0A2M9BCQ1	113 (10.80)	204 (19.50)	729 (69.69)

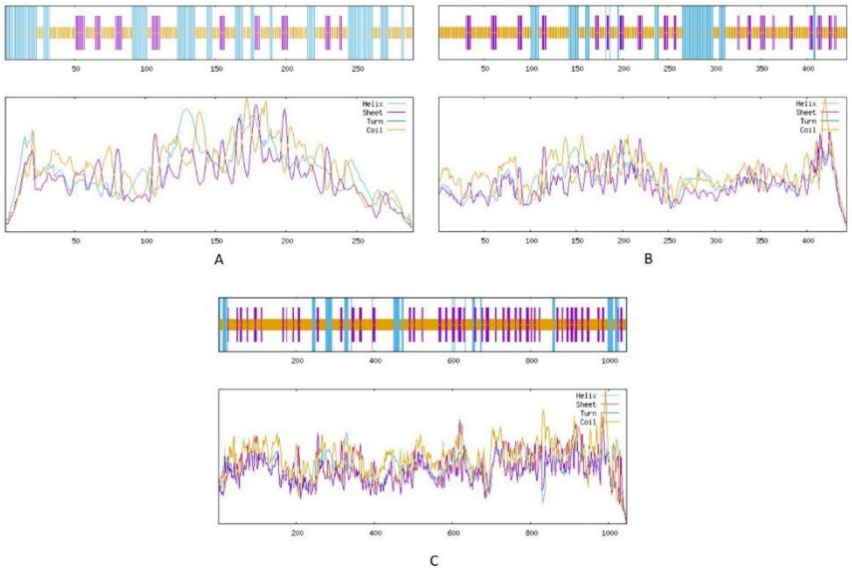

Figure 1: Predicted of secondary (2D) configuration of PETases documented in current study, using SOPMA tool

3.2 Prediction of 3D configuration of PETases

The most complex folding was observed in enzyme associated with *C. suwonensis*, followed by *S. labedae* (Figure 2).

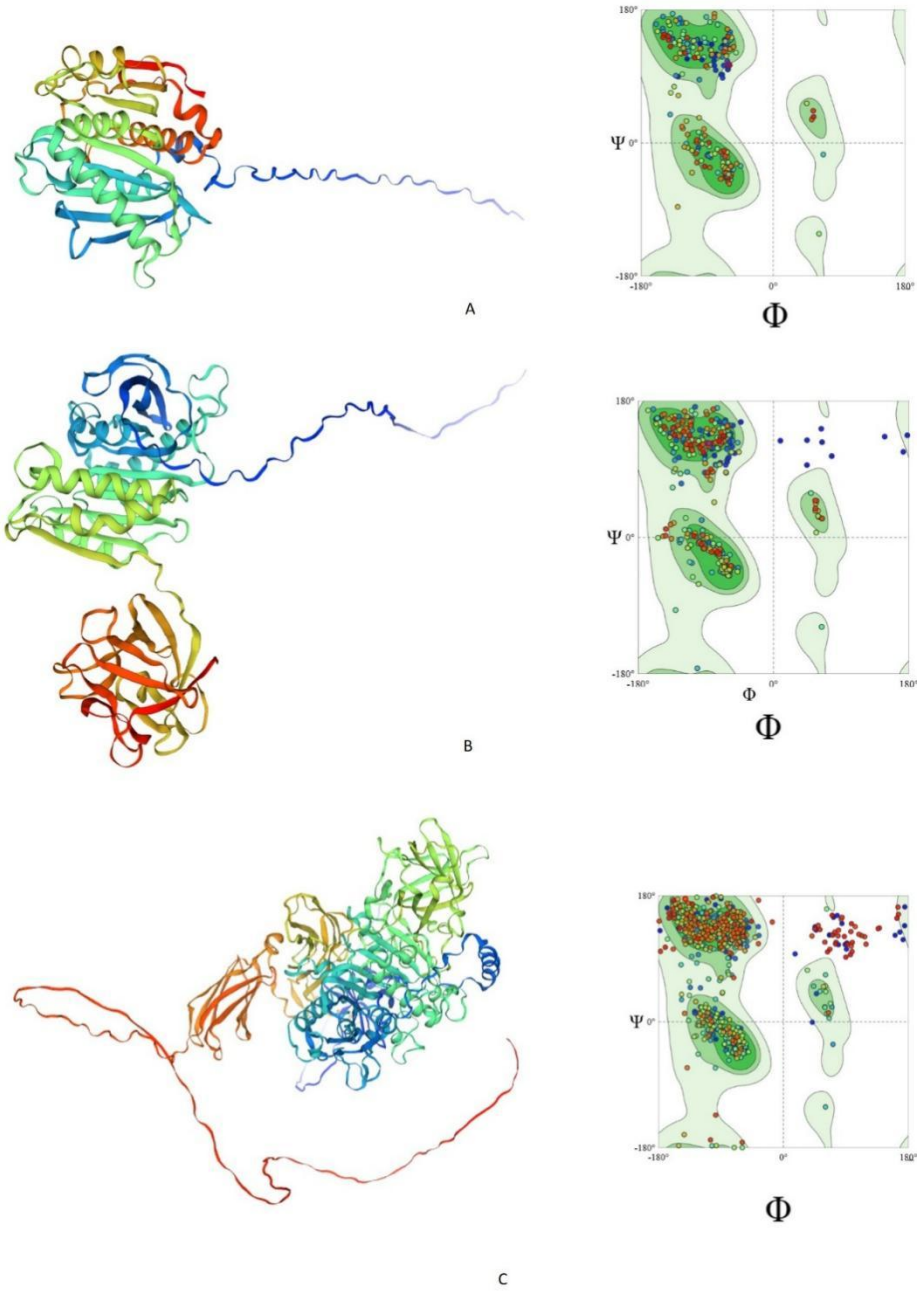

Figure 2: Prediction of tertiary (3D) configuration of PETases via SWISS-MODEL tool and Ramachandran plots, documented in current study

A: A0A0K8P6T7, B: A0ABP6QVC8, C: A0A2M9BCQ1

Assessment of quality of predicted configuration via Ramachandran plot documented MolProbity and clash scores, Ramachandran outliers and favoured, Rotamer outliers, C-beta deviations, Bad bonds and bad angles for structure of enzyme associated with *P. sakaiensis* as 1.27, 0.24, 0.00%, 93.75%, 2.21%, 1, 0/2170 and 5/2956, respectively. In case of *S. labedae* PETase, Clash and MolProbity scores (0.93 and 1.27, respectively), Ramachandran outliers (2.04%) and favoured (92.06%), Rotamer outliers (0.60%), bad bonds (0/3352) and angles (33/4597), C-beta deviations (2), cis-prolines (1/27), cis non-prolines (4/415, twisted prolines (1/27) and twisted non-prolines (9/415) were recorded. In *C. suwonensis* PETase, values recorded for MolProbity score, Clash score, Rotamer outliers, Ramachandran favoured, C-beta deviations, bad angles, bad bonds, cis prolines and non-prolines and twisted prolines and non-prolines were measured as1.54, 0.27, 2.69%,

87.55%, 41, 168/10718, 4/7816, 6/79 and 13/966 and 5/79 and 55/966, respectively.

3.3 Prediction of conserved motifs of PETases

Total eleven conserved domains were identified for A0A0K8P6T7. The relative entropy and Bayes threshold of these motifs was calculated as 24 and 6.20945 for IFACEN, TFACEN, QFLEIN, 31 and 6.27819 for WMKRFMDN and WGPRLASH, 23.6 and 7.14466 for PIYGKV and PIYDSM, 25.8 and 7.13955 for RMGVMGW and RQSSIKW and 25.1 and 7.13955 for LRQVASL and LMQAAVL, respectively.

Relative entropy and Bayes threshold for eleven motifs of A0ABP6QVC8 were recorded as LRSRANNQYVCAENAGTDPLIANRTAIGDWER FDLID, LRARVNGRYVCAENAGANALIANRTAVGPWE TFQRVN and LRAQANNLYVSAGNAGTAPLIANRTTIGPSESFD LIN (128.4 and 7.52356, respectively), VDWMRWSLY and BDQYIWSGY (38 and 6.6127, respectively), ICEFSH and KVLFVH (26.7 and 7.76818, respectively), DPFAGH and KAFLTH (22.7 and 7.76818, respectively) and CVDMGNPSS and DVYNGNQSM (35.3 and 6.6127, respectively).

In case of A0A2M9BCQ1, the motifs and their relative entropy and bayes threshold determined was 23.7 and 8.8832 for EQPYCV, ILPYCD and TYQYCA, 81.5 and 8.99859 for FLHGRHTSCSGGTRNPDSWPCN and FFNTSWTPSKYAFSDDWNNN, 34.2 and 9.0182 for NQQGQHFF and EQQFLPMF, 44 and 9.01402 for MPKSVRHWKHI and LRKVLLQQLTI and 25.1 and 9.02098 for VMGHSR and VMGANH, respectively. Motifs found in all isoforms of enzyme are shown in Figure 3 and Table 3.

Figure 3: Prediction of conserved motifs of PETases based on MEME suite

A: A0A0K8P6T7, B: A0ABP6QVC8, C: A0A2M9BCQ1

Table 3: *Prediction of conserved motifs of PETases through MEME suite*

E-value	p-value	motif
A0A0K8P6T7		
9.8e-002	4.51e-9	NFSSVTVPTL IFACEN DSIAPVNSSA
	1.05e-8	RFMDNDTRYS TFACEN PNSTRVSDFR
	1.01e-5	IYDSMSRNAK QFLEIN GGSHSCANSG

4.7e+001	8.28e-12	QALIGKKGVA WMKRFMDN DTRYSTFACE
	4.16e-9	YTARQSSIKW WGPRLASH GFVVITIDTN
1.2e+002	2.14e-8	VASLNGTSSS PIYGKV DTARMGVMGW
	6.24e-8	SIAPVNSSAL PIYDSM SRNAKQFLEI
1.9e+002	6.62e-9	SPIYGKVDTA RMGVMGW SMGGGGSLIS
	2.70e-8	AIAIVPGYTA RQSSIKW WGPRLASHGF
7.0e+002	1.39e-8	SSRSSQQMAA LRQVASL NGTSSSPIYG
	1.71e-8	MNFPRASR LMQAAVL GGLMAVSAAA
A0ABP6QVC8		
2.7e-012	1.49e-42	TPLPTGPIV LRSRANNQYVCAENAGTDPLIANRTAIGDWERF LGGGAV S DLID A
	2.91e-35	LIDLGGGAV LRARVNGRYVCAENAGANALIANRTAVGPWETF NSDGT A QRVN A
	1.59e-33	RVNNSDGTTS LRAQANNLYVSAGNAGTAPLIANRTTIGPSESFDLIN G
6.4e-001	7.00e-13	YNHTQTVATY VDWMRWSLY ADTAARDRLP
	1.03e-11	KAFLTHRGTG HDQYIWSGY NHTQTVATYV
1.3e+000	2.56e-9	NAPIADGVFP ICEFSH GLGGNPESYA
	9.27e-9	MGNPSSTVAA KVLFVH GDQDTLTQYS
8.9e+001	8.79e-8	NTLALNTTAG DPFAGH LDTTTGVGVA
	1.01e-7	RQAYGELPAP KAFLTH RGTGHDQYIW
1.1e+002	3.38e-12	SRIAAAVPFA CVDMGNPSS TVAAKVLFVH
	5.09e-11	TSSGTQGNVQ DVYNGNQSM DVSQVITNTL
A0A2M9BCQ1		
6.1e+000	7.13e-9	ASLSARPVPG EQPYCV NSLTTSQVPD
	2.65e-8	MTLPDVPTAT ILPYCD GDVSNQQGQH
	1.57e-7	DTDVRVLGAG TYQYCA SLSARPVPGE
6.8e+000	8.56e-25	GAAGERPVVL FLHGRHTSCSGGTRNPDSWPCN PDQVDVASYL
	5.48e-23	SVLVMGANHN FFNTSWTPSKYAFSASDDWNNN NGDPDCKYGV
1.4e+001	1.21e-11	ILPYCDGDVS NQQGQHFF DDSQHAFDDN
	2.48e-11	TAFFRLTMGD EQQFLPMF DGSDAKPVSA
9.0e+000	1.50e-16	MPKSVRHWKHI ALATALCFAA
	1.58e-11	LTVLPGTVSP LRKVLLQQLTI PTGDFAGVNL
2.5e+002	6.96e-9	KGRLDLDHVG VMGHSR GGDGAVRAAL
	1.06e-8	DDNVLRSSVL VMGANH NFFNTSWTPS

4. Discussion

Utilization of PET-eating enzymes is an emerging and sustainable strategy to combat the plastic accumulation problem (Patel and Lee 2022). According to the previously published work, higher temperature enhances the PET degradation rate. PET hydrolysis is carried out at 65-70°C in aqueous solution (Kawai et al. 2019). Another study has documented modification of enzyme through addition of hydrogen bonds via mutations (S121E and D186H) which enhanced thermal stability of enzyme (Riaz et al. 2025). In current study, we identified highest content of random coil followed by alpha helix. Higher the random coil, greater is the thermal instability. While increase in alpha helix enhances temperature resistance in enzyme. So, we may manipulate the enzyme to increase the alpha helix content, thereby increasing thermal stability and in turn PET hydrolysis rate.

Conserved motifs of PET hydrolases have been explored previously that play direct role in binding of enzyme with PET for the cleavage of ester bond (Han et al. 2024; Ogunlusi et al. 2025). These motifs include, Ser-His-Asp triad, G-X-S-X-G motif,

hydrophobic domains comprising of tryptophan and tyrosine and disulfide bridges (IsPETase associated Cys239-Cys277) (Jin and Jia 2024; Zhang et al. 2022). Finding the conserved domains shows consistency in current project and previous literature (Saini et al. 2023). The characteristics explored in this study might be of great significance for engineering of wild type PETases to get optimum PET bioremediation.

Statements and Declarations

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Ethical approval: N/A

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Data availability statement: The amino acid sequence of PETases isoforms documented in current study is available at UNIPROT database (https://www.uniprot.org) under Accession numbers A0A0K8P6T7, A0ABP6QVC8 and A0A2M9BCQ1

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