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Original Article

In silico identification of potential polyethylene terephthalate-degrading enzymes

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ABSTRACT

Plastic usage has increased dramatically in recent years in which polyethylene terephthalate (PET) is one of the most abundant types of plastic waste. Researchers had discovered a number of microorganisms that have plastics degrading enzymes, and *Ideonella sakaiensis* is one of the most studied examples of an organism that has a PET-degrading enzyme. These well-known enzymes degrade PET at a moderate turnover rate, though. Therefore, more study is required to improve the variety of microbes and enzymes that can break down plastic. In this study, molecular docking analysis was used to find the IsPETase homologue enzymes that can bind to the PET ligand. Their capacity to bind to PET indicates that it might be a potential enzyme that breaks down PET. The molecular docking predicted a novel alpha/beta hydrolase (7CUV and 7E31) as the most potential candidate enzyme for PET-degrading activity (ΔG value: -6.8 kcal/mol and -5.2 kcal/mol, respectively). With ΔG values of -5.5 kcal/mol and -5.7 kcal/mol, respectively, an esterase with a long N-terminal extension (LNTE) isolated from a leaf-branch compost (3WYD) and a lipase referred to as SeL from *Streptomyces exfoliatus* (1JFR) also demonstrated a promising binding affinity to PET. In conclusion, computational techniques such as *in silico* molecular docking analysis, in conjunction with the expanding databases of enzyme and genomic information, offer the potential to quickly uncover new enzymes and microorganisms with the capacity to breakdown PET. To further confirm these possible enzymes, lab-based methods such as biochemical, structural, and others are required.

KEYWORDS: *in silico*, molecular docking, plastic-degrading bacteria, polyethylene terephthalate (PET).

INTRODUCTION

Plastic is a synthetic organic polymer that is typically cheap to manufacture and therefore is common to be produced in large scale. The primary production of plastics had significantly risen from 23 million tons (Mt) in 1967 to 407 Mt in 2015 [1]. This increase is due to plastic's desirable characteristics such as being resilient, non-reactive and chemically resistant, which also cause them to degrade slowly [2]. Billions of tons of plastic are accumulated in the environment due to poor recycling and low circular use. This caused living organisms to accidentally digest the plastic, contaminating the food chain and causing health issues [1]. The primary source of plastic trash is packaging waste, with polyethylene terephthalate (PET) being one of the most prevalent forms of plastic waste [3].

Currently, UV radiation and mechanical disruptions are the two most common methods of plastic degradation [4]. However, according to recent studies, the majority of plastic trash is actually more likely to disintegrate into tiny bits

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of microplastic than to degrade [5]. Microplastics need to be biodegraded to be eventually transformed into harmless products that can be reutilized by nature [2]. Researchers had found several microorganisms that possess plastic degrading enzymes, which mostly act on PET or ester-based polyurethane (PUR) [4]. To date, *Ideonella sakaiensis* PETase is one of the well-studied PET degrading enzyme [6]. Nevertheless, these well-known enzymes have average turnover rates; therefore, more study is required to improve the variety of microbes and enzymes that can break down plastic.

A few methods have been widely used in identifying plastic-degrading enzymes: pure-culturing from environmental samples [6, 7], metagenomics [8], and *in silico* computational methods [9]. In contrast to the first two methods, the *in silico* method enables rapid discovery of hypothetical plastic-degrading enzymes homologous to known proteins. Duru *et al*. [10] reported a high affinity of *Is*PETase on polycarbonate *via* an *in silico* analysis, indicating that enzyme might be just as effective at breaking down polycarbonate as PET. Based on *in silico* screening. *Almeida et al.* [9] were successful in finding a new PETase-like enzyme, and its plastic-degrading activity was validated experimentally.

In this study, an *in-silico* search was conducted to identify enzymes that are homologous to *I. sakaiensis*'*s* PET hydrolases, and their PET binding affinity was further assessed using molecular docking. We believe that this study would successfully increase the diversity of putative PET-degrading proteins.

METHODS

Potential *Is*PETase-like protein homology search

Amino acid sequence of *I. sakaiensis* PETase (GAP38373) was retrieved from NCBI database (https://www.ncbi.nlm.nih.gov/) and used as the target protein. The sequence was searched against Protein Data Bank (PDB) database using protein-protein PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool) alignment program. Candidates (maximum subject sequence length of 400 aa) with the e-values below $1e^{-04}$ were shortlisted for molecular docking analysis.

Preparation of PET ligand

The 3D structure data file (SDF) of PET ligand compounds was downloaded from PubChem database. The ligand file format was converted into pdbqt file using PyMOL v2.5.2 and AutoDock Tools v1.5.7 [11].

Preparation of homologous protein(s)

The 3D structure data files of *I. sakaiensis*'s PET hydrolase and the shortlisted homologous proteins were retrieved from PDB database. The active sites of each protein were predicted using Biovia Discovery studio v21. The removal of interfering water molecules and the addition of missing atoms were performed using AutoDock Tools to increase the docking accuracy.

Molecular docking study of potential protein-ligand binding

The docking of the homologous proteins on PET ligand was performed using Autodock Vina with exhautiveness of 16. The grid box size was set to the dimension size x: 30, size y: 30, size z: 30 and spacing angstrom was set to 1.000. The center grid box was set according to the predicted active site of each enzyme. The results in terms of binding free energy (ΔG Energy (Kcal/mol)) for each homologous protein were obtained and compared to the positive control, which was *I. sakaiensis*'s PET hydrolase.

RESULTS

Screening for enzymes homologous to *Is*PETase

A PSI-BLAST was performed against the PDB database to search for proteins that are homologous to *Is*PETase. PSI-BLAST is suitable for the detection of functional homologues even in proteins that are distantly related to the target protein. The PDB was chosen as the database to ensure all candidates have experimentally verified structures for molecular docking analysis. With an E-value cut-off of $1e^{-04}$, thirty-nine proteins were shortlisted as the homologous enzymes of *Is*PETase (Table 1). Based on the conserved sequences between the homologous enzymes and *Is*PETase, they are hypothesized to be involved in PET degradation.

Table 1. PSI-BLAST identification of *I. sakaiensis* PETase's homologous enzymes.

No	PDB ID	Length (aa)	Query cover (%)	Percent identity (%)	E-value
1	7DZU	268	84	81.63	9e-127
2	7DZT	268	84	81.22	3e-126
3	7DZV	268	84	81.22	4e-126
4	7CWQ	270	89	63.43	5e-115
5	7ECB	294	88	52.45	2e-86
6	7EC8	294	88	53.96	3e-86
7	5LUI	265	88	51.71	6e-84
8	5ZOA	261	88	51.71	7e-84
9	6SCD	312	99	48.20	8e-84
10	6SBN	312	99	47.87	5e-83
11	5LUL	265	88	50.95	6e-83
12	5LUK	265	88	50.57	2e-82
13	5LUJ	265	88	50.19	1e-81
14	6AID	278	87	50.19	3e-78
15	3VIS	306	87	50.19	4e-78
16	5ZRQ	265	89	45.42	4e-76
17	5ZNO	265	89	45.42	5e-76
18	7CEH	262	89	45.42	6e-76
19	4CG1	282	88	51.71	5e-75
20	4EB0	258	89	49.05	1e-74
21	4CG3	313	88	51.71	1e-74
22	6THS	258	89	48.67	3e-74
23	7CTS	263	88	45.38	3e-74
24	7W44	269	89	48.67	1e.73
25	7W1N	270	89	48.67	5e-73
26	7W45	270	89	48.29	5e-73
27	7E31	258	89	48.86	6e-73
28	6THT	258	89	48.29	1e-72
29	7VVE	260	89	48.29	1e-72
30	7VVC	270	89	48.29	1e-72
31	7DS7	277	89	48.29	2e-72
32	7NEI	267	88	48.28	3e-72
33	7CUV	258	88	48.28	3e-72
34	7CEF	262	89	45.80	5e-68

Table 1 continued..

No	PDB ID	Length (aa)	Query cover (%)	Percent identity (%)	E-value
35	4WFI	273	88	45.59	3e-67
36	7CTR	263	88	45.77	3e-66
37	1JFR	262	88	47.51	2e-65
38	7PZJ	366	84	38.49	8e-44
39	3WYD	228	31	31.18	1e-04

Molecular docking analysis of enzymes homologous to *Is*PETase

All homologous enzymes, with the exception of 4CG3, were subjected to molecular docking analysis using Autodock Vina. 4CG3 was not included as its structure format was incompatible for Autodock Vina analysis. Table 2 summarizes the binding affinity of the thirty-eight proteins homologous to PET ligand, alongside with IsPETase that served as the positive control. IsPETase demonstrated a free binding energy, Δ*G*, of -5.3 kcal/mol, which is in line with the Δ*G* value previously reported by Duru *et al*. [10]. It was hypothesized that homologous enzymes with a Δ*G* value comparable to or lower than IsPETase would have a strong affinity for PET ligand and could effectively metabolise PET. Based on this premise, twenty-four enzymes, predominantly from *Thermobifida sp.* and *Saccharomonospora sp.*, were identified (Table 2). All enzymes, with the exception of four, were known to be capable of degrading PET. The evidence of each enzyme in PET degradation was sourced and well referenced in Table 2.

DISCUSSION

The majority of enzymes with a Δ*G* similar to *Is*PETase have already been identified as plastic-degrading enzymes, demonstrating the validity of an *in-silico* analysis for making solid predictions. The enzymes shortlisted in Table 2 are predominantly cutinases from *Thermobifida* and *Saccharomonospora* species. PETase and cutinases share high sequence identity [29]; this explains why many cutinases were identified from the PSI-BLAST search. *T. fusca* cutinase is one of the most thoroughly investigated PET-degradation enzymes that has the potential to be used in bio-cycling [30]. Cutinases from other *Thermobifida* species, like *T. alba* and *T. cellulosilytica,* are also demonstrating a potential PET-degradation activity [31]. On the other hand, enzymes known to be able to degrade PET, such as esterases and lipases, were also discovered. This evidence suggests that a computational prediction that involves IsPETase homologous search and molecular docking may discover novel enzymes that could similarly breakdown PET.

Among the enzymes with Δ*G* less than 5.0 kcal/mol, four enzymes (3WYD, 1JFR, 7CUV, and 7E31) were found with no evidence of PET biodegradation. The low Δ*G* values point to these enzymes' great affinity for the PET ligand, indicating that they may be able to break down PET. 3WYD was identified from a leaf-branch compost metagenome [32]. Plant compost is an ideal source for polyester-hydrolyzing enzymes because it serves as a habitat for thermophilic microorganisms that are good at breaking down plant polymers like cutin [12]. Additionally, 3WYD is an esterase, and it has been discovered that esterases from various microorganisms are capable of cleaving the ester bond between PET monomers [31]. For example, nitrobenzylesterase (BsEstB) from *Bacillus subtilis* is capable of hydrolyzing PET into terephthalic acid (TPA) and MHET [mono(2-hydroxyethyl)] TPA [33]. Sequence analysis conducted by Okano *et al*. [32] revealed that 3WYD shared a 46% identity with *Candidatus Solibacter usitatus* and its C-terminal esterase domain showed a relatively high amino acid sequence identity with the thermostable esterase EstA from *Thermotoga maritima,* indicating

Table 2. Binding free energy of PET ligand on the homologous proteins of *Is*PETase.

No	PDB ID	Resolution (Å)	Protein description	Enzyme classification	Organism	-Δ*G* Energy (Kcal/mol)	Evidence on PET degradation	References
1	7NEI	1.30	Polyester hydrolase Leipzig 7 (PHL-7)	Hydrolase	Unidentified unclassified sequences	-7.1	Yes	[12]
2	7CUV	1.45	Alpha/beta hydrolase	Hydrolase	Artificial sequences	-6.8	No	Not found
3	7CWQ	1.65	Cutinase	Hydrolase	*Burkholderiales bacterium*	-6.5	Yes	[13]
4	7CEF	1.60	Mutant PET-degrading cutinase Cut190/ S226P/R228S	Hydrolase	*Saccharomonospora viridis*	-6.5	Yes	[14]
5	7VVC	1.82	Inactive mutant of leaf-branch compost cutinase	Hydrolase	Uncultured bacterium	-6.4	Yes	[15]
6	3VIS	1.76	Cutinase Est119	Hydrolase	*Thermobifida alba*	-6.1	Yes	[16]
7	5ZOA	1.54	Cutinase	Hydrolase	*Thermobifida fusca*	-5.9	Yes	[17]
8	5ZRQ	1.12	PET-degrading cutinase Cut190 S176A/S226P/R228S	Hydrolase	*Saccharomonospora viridis*	-5.7	Yes	[18]
9	5ZNO	1.60	PET-degrading cutinase Cut190 S176A/S226P/R228S	Hydrolase	*Saccharomonospora viridis*	-5.7	Yes	[18]
10	1JFR	1.90	Lipase	Serine Hydrolase	*Streptomyces exfoliates*	-5.7	No	Not found
11	7DZT	2.35	PETase	Hydrolase	*Rhizobacter gummiphilus*	-5.6	Yes	[19]
12	7EC8	1.35	Lipase PET2 mutant	Hydrolase	Uncultured bacterium	-5.6	Yes	[20]
13	5LUL	1.90	A triple variant of cutinase 2	Hydrolase	*Thermobifida cellulosilytica*	-5.6	Yes	[21]

Table 2 continued..

No	PDB ID	Resolution (Å)	Protein description	Enzyme classification	Organism	-Δ*G* Energy (Kcal/mol)	Evidence on PET degradation	References
14	7vve	1.98	Leaf-branch compost cutinase variant	Hydrolase	Uncultured bacterium	-5.6	Yes	[15]
15	3WYD	1.53	C-terminal esterase domain of LC-Est1	Hydrolase	Uncultured Organism	-5.5	No	Not found
16	7w45	1.94	A leaf-branch compost cutinase variant LCC ICCG_KIP	Hydrolase	Uncultured bacterium	-5.4	Yes	[15]
17	6SCD	1.35	Polyester hydrolase PE-H Y250S mutant	Hydrolase	*Halopseudomonas aestusnigri*	-5.4	Yes	[22]
18	6ANE*	2.02	PET Hydrolase	Hydrolase	*Ideonella sakaiensis*	-5.3	Yes	[6]
19	7pzj	2.10	Bacteroidetal (PET) esterase	Hydrolase	*Kaistella jeonii*	-5.3	Yes	[23]
20	5LUK	1.45	A double variant of cutinase 2	Hydrolase	*Thermobifida cellulosilytica*	-5.3	Yes	[21]
21	7E31	1.38	Alpha/beta hydrolase mutant	Hydrolase	Unidentified unclassified sequences	-5.2	No	Not found
22	4CG1	1.40	Thermostable PET degrading hydrolase	Hydrolase	*Thermobifida fusca*	-5.2	Yes	[24]
23	7CEH	1.09	Mutant PET-degrading cutinase Cut190 S176A/S226P/R228S	Hydrolase	*Saccharomonospora viridis*	-5.1	Yes	[14]
24	6SBN	1.09	Polyester hydrolase PE-H	Hydrolase	*Halopseudomonas aestusnigri*	-5.1	Yes	[22]
25	5LUJ	2.20	Cutinase 2	Hydrolase	*Thermobifida cellulosilytica*	-5.0	Yes	[21]
26	7DZU	2.40	PETase K169A mutant	Hydrolase	*Rhizobacter gummiphilus*	-4.8	Yes	[19]

Table 2 continued..

No	PDB ID	Resolution (Å)	Protein description	Enzyme classification	Organism	-Δ*G* Energy (Kcal/mol)	Evidence on PET degradation	References
27	7DZV	1.60	PETase E186A mutant	Hydrolase	*Rhizobacter gummiphilus*	-4.8	Yes	[19]
28	6THS	1.10	Leaf-branch cutinase S165A variant	Hydrolase	Uncultured bacterium	-4.8	Yes	[25]
29	6THT	1.14	Leaf-branch compost cutinase quintuple variant	Hydrolase	Uncultured bacterium	-4.8	Yes	[25]
30	4WFI	1.45	PET-degrading cutinase Cut190 S226P mutant	Hydrolase	*Saccharomonospora viridis*	-4.8	Yes	[26]
31	7w44	1.85	A leaf-branch compost cutinase variant LCC ICCG_RIP	Hydrolase	Uncultured bacterium	-4.7	Yes	[15]
32	7w1n	1.88	A leaf-branch compost cutinase variant LCC ICCG_KRP	Hydrolase	Uncultured bacterium	-4.5	Yes	[15]
33	5LUI	1.50	Cutinase 1	Hydrolase	*Thermobifida cellulosilytica*	-4.5	Yes	[21]
34	6AID	1.30	Polylactate degrading cutinase	Hydrolase	*Thermobifida alba*	-4.5	Yes	[27]
35	7CTR	1.20	Mutant PET-degrading cutinase Cut190	Hydrolase	*Saccharomonospora viridis*	-4.5	Yes	[28]
36	7DS7	2.15	Leaf-branch compost cutinase from Biortus	Hydrolase	Unidentified prokaryotic organism	-4.4	No	Not found
37	7ECB	1.83	Polyethylene terephthalate hydrolyzing lipase PET2 mutant	Hydrolase	Uncultured bacterium Bacteria	-4.1	Yes	[20]

Table 2 continued..

No	PDB ID	Resolution (Å)	Protein description	Enzyme classification	Organism	-Δ*G* Energy (Kcal/mol)	Evidence on PET degradation	References
38	4EB0	1.50	Leaf-branch compost bacterial cutinase homolog	Hydrolase	Uncultured bacterium Bacteria	-4.1	No	Not found
39	7CTS	1.10	Mutant PET-degrading cutinase Cut190	Hydrolase	*Saccharomonospora viridis*	-4.0	Yes	[28]

*PETase from *I. sakaiensis* was used as a positive control.

that 3WYD is potentially a more thermal stable PET-degrading enzyme than the heat-labile IsPETase [6].

IJFR, on the other hand, is a lipase from *S. exfoliatus* [34]. Lipase has a high affinity for water-insoluble substrates, making it a good candidate to hydrolyze hydrophobic PET [32]. *Thermomyces lanuginosus* lipase, which has been reported to be able to hydrolyze the PET into TA and MHET [35], is one example of lipase known to degrade PET. Although not having been confirmed as a PET-degrading enzyme, IJFR has been used as a template for homology modelling of *Thermobifida* cutinases, which is known to be able to degrade PET [36]. Additionally, it shared a putative catalytic triad with the enzyme from *T. alba*, which was demonstrated to have the ability to degrade PET polymer [7]. Furthermore, another *Streptomyces* species able to degrade PET has been reported [37]. As species within the same genus share a high degree of similarity in substrate affinities and catalytic properties [38], *S. exfoliatus* is highly likely a candidate for PET degradation.

7CUV, a novel αβ-hydrolase, is yet another new enzyme discovered from this study (Gao *et al*., unpublished). As of this writing, there is no published information on 7CUV and its mutant, 7E31, the fourth enzyme discovered in this study (Gao *et al*., unpublished). Nevertheless, several αβ-hydrolases have been identified as PET-degrading enzymes. These enzymes include carboxylic ester hydrolase (PE-H) from *P. aestusnigri* VGXO14T [22], a lipase (Cut190) from *S. viridis* AHK190 [26], and a cutinase (Est119) from *T. alba* AHK119 that displayed an overall structure of typical αβ-hydrolase fold [16].

CONCLUSION

With the use of computational predictions that include homologous identification and molecular docking, this study has identified four enzymes with previously unknown PET-degrading abilities. The ΔG values resulted from molecular docking were comparable to or lower than *I. sakaiensis*'s PET hydrolase, suggesting that the enzymes were able to form a stable complex with PET ligand and metabolize it. The four enzymes include a novel αβ-hydrolase and its mutant form, an esterase with a long N-terminal extension isolated from a leaf-branch compost, and a lipase termed SeL from *S. exfoliatus*. To further confirm the expected roles of the enzymes discovered using *in silico* methods, lab-based techniques like heterologous expression of the predicted enzymes in an expression host should also be done.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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