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REVIEW ARTICLE

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The diversity of PET degrading enzymes: A systematic review of sequence, structure, and function

Nitay Ahituv | Dekel Freund | Raul Mireles | Lianet Noda-García

Institute of Environmental Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

Correspondence
Lianet Noda-García, Institute of Environmental Sciences, The Hebrew University of Jerusalem, Herzl 229, Rehovot 7610001, Israel.
Email: lianet.noda@mail.huji.ac.il

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Abstract
Polyethylene terephthalate (PET) is one of the most significant plastic pollutants. Unlike other plastic polymers, PET can be degraded by PET-hydrolytic enzymes (PETases). Over the past two decades, numerous publications have reported the discovery, characterization, and engineering of PETases. This review thoroughly examines the sequence, structure, and functional diversity of naturally occurring PETases. To achieve this, we compiled data from 48 publications into a single table. The resulting dataset enabled us to contextualize previously reported features and shed light on the sequence–structure–function relationships of PETases. Finally, we review selected engineering campaigns and suggest future directions for the enzymatic recycling of PET under mesophilic and thermophilic conditions, aiming to understand the gaps to tackle the PET pollution crisis.

KEYWORDS
dataset, PET biodegradation, PET-hydrolytic enzymes (PETase), polyethylene terephthalate, recycling

1 | INTRODUCTION

Plastics have become ubiquitous in human life due to their durability, flexibility, and inexpensive production. Although introduced only around 70 years ago (Geyer et al., 2017), today plastic contaminates every natural habitat—from the tallest mountains (Napper et al., 2020) to the deepest parts of the oceans (Chiba et al., 2018), as well as our food and our bodies (Toussaint et al., 2019). Global plastics production has now exceeded 400 million metric tons (MT) annually. Further, it is expected to reach 700 million MT by 2030, which equates to around 80 kg of plastic produced per person on Earth (Britt et al., 2019). Clearly, the impact on the environment will only worsen without recycling.

Polyethylene terephthalate (PET) is a plastic composed of two fossil-fuel-based monomers, ethylene glycol (EG) and terephthalic acid (TPA), linked by ester bonds (Pang et al., 2016). PET accounted for approximately 10% of the total plastic production by mass between 2002 and 2014, making it the fourth most produced plastic (Geyer et al., 2017; Stubbins et al., 2021). PET is primarily used for food packaging, bottles, and fibers (Welle, 2011). It accounted for 44.7% of single-serve beverage packaging in the United States in 2021 (Benyathiar et al., 2022). Recently, governmental awareness of the urgency of plastic recycling, for example, the 2018 European Strategy for Plastics in a Circular Economy, encouraged taxing non-recycled plastics (Pazienza & De Lucia, 2020), increasing the demand for recycled PET (Tournier et al., 2023).

The most widely used process, thermal recycling of PET, involves melting and reshaping the polymer (Altun & Ulcay, 2004). This process, however, has a crucial limitation: PET can only endure a certain number of cycles before it needs to be downcycled into lower-value products like fibers, which are not

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recyclable (Tournier et al., 2023). In contrast to thermal recycling, enzymatic degradation breaks the ester bonds linking PET monomers, which can then be purified and polymerized into new PET without losing quality (Uekert et al., 2022). Thus, theoretically, enzymatic PET recycling could be repeated infinitely without compromising the plastic's quality and functionality.

In 2005, a cutinase from *Thermobifida fusca* was reported as the first enzyme capable of effectively hydrolyzing PET film (Müller et al., 2005). Over the past 20 years, numerous PET-degrading enzymes (PETases) have been discovered and engineered (Arnal et al., 2023; Duan et al., 2023; Satta et al., 2024). Additionally, organisms such as *Ideonella sakaiensis* (Yoshida et al., 2016) have been found to degrade and utilize PET as a carbon source, thereby opening up the possibility for cell-based catalysis. With the launch of Carbios—the first commercial company focused on enzymatic PET recycling—the field has begun transitioning from research to industrial application (Johnson, 2024; Tournier et al., 2020). Carbios's patent portfolio covers key aspects of PET bio-recycling at an industrial scale, including proprietary enzyme cocktails (Boisart & Maille, 2018), multi-enzyme degradation strategies (Guemard & Dalibey, 2022), synergistic surface-binding protein combinations (David et al., 2020), and substrate pretreatment processes that enhance enzymatic PET hydrolysis (Desrousseaux et al., 2022). Some challenges, however, still hinder the adoption of biodegradation as a viable strategy to mitigate PET pollution, namely, the degradation of crystalline PET and the development of even more efficient and stable PETases than currently available.

The growing potential of enzymatic recycling and the pressing problem of PET pollution have spurred scientific research; more than 250 publications regarding PETases have been published in PubMed in the last 5 years. This abundance of studies highlights the need for a comprehensive review, which might aid in shaping the future of research in this area and directing resources effectively to advance PET biodegradation. Previous research articles and reviews have examined various aspects of the sequence and structural diversity of a subset of PETases (Duan et al., 2023; Erickson et al., 2022; Khairul Anuar et al., 2022; Liu et al., 2023; Wei et al., 2022); in 2022, the Plastics-Active Enzymes (PAZy) database was created, containing sequence and taxonomy information of plastic-degrading enzymes, including PETases (Buchholz et al., 2022). However, a systematic analysis that includes function, beyond sequence and structure, is still missing.

To achieve this, we created the PETase Activity Natural Dataset (PANDA), which contains the functional information of 99 different naturally occurring PETases (up to October 2024) and one engineered enzyme (LCC_ICCG), a benchmark in the field (Erickson et al., 2022; Makryniotis et al., 2023; Tournier et al., 2020) (Supplementary Table 1). In this review, any enzyme degrading PET analogs with two or more TPA moieties is referred to as a PETase, regardless of whether PET is the enzyme's natural or preferred substrate, or the efficiency of degradation. Five enzymes in PANDA do not include sequence and structure information, as they are not publicly available. We excluded enzymes whose activity was not tested when pure. In this way, each record in PANDA represents a pure PETase's biochemical activity in one experiment, resulting in 244 records from 48 publications (Arnling Bååth et al., 2022; Avilan et al., 2023; Billig et al., 2010; Blázquez-Sánchez et al., 2022; Bollinger et al., 2020; Brackmann et al., 2023; Brinch-Pedersen et al., 2024; Carr et al., 2023; C.-C. Chen et al., 2021; Danso et al., 2018; Distaso et al., 2023; Edwards et al., 2022; Effendi et al., 2024; Eiamthong et al., 2022; Erickson et al., 2022; Guo et al., 2023; Han et al., 2024; Herrero Acero et al., 2011; Hong et al., 2023; Jabloune et al., 2020; Lee et al., 2024; Li et al., 2022; Mabashi-Asazuma et al., 2024; Makryniotis et al., 2023; Mamtimin et al., 2024; Meyer Cifuentes et al., 2022; Müller et al., 2005; Perez-Garcia et al., 2023; Perz et al., 2016; Qi et al., 2023; Ribitsch et al., 2011; Ribitsch, Herrero Acero, Greimel, Dellacher, et al., 2012; Ribitsch, Herrero Acero, Greimel, Eiteljoerg, et al., 2012; Ronkvist et al., 2009; Sagong et al., 2021; Seo et al., 2025; Sonnendecker et al., 2022; Sulaiman et al., 2012; Taxeidis et al., 2024; Tiong et al., 2023; Vázquez-Alcántara et al., 2021; Vidal et al., 2024; Wei et al., 2014; Xi et al., 2021; Yoshida et al., 2016; Zhang et al., 2022, 2024; Zhou et al., 2024). This information allowed us to summarize previous concepts and shed light on PETases' sequence, structure, and functional diversity. See Supplementary Texts 1–3 and Supplementary Table 2 for further details on data collection and processing. Finally, we provide a brief overview of the current status of engineered PETases within mesophilic and thermophilic ranges, examining the industrial potential of each approach. Other reviews focusing mainly on engineered enzymes can also be checked (Barclay & Acharya, 2023; Gao et al., 2021; Kawai et al., 2024; Liu et al., 2023; Radley et al., 2023; Samak et al., 2020; Wei et al., 2022; Yao et al., 2024).

2 | PET-DEGRADING ACTIVITY IS ACCESSIBLE FROM DIVERSE SEQUENCE BACKGROUNDS

The amino acid sequence diversity of PETases has been assessed using sequence similarity networks (SSNs) (Erickson et al., 2022; Perez-Garcia et al., 2023; Qi et al., 2023; Seo et al., 2025) and phylogenetic trees (Erickson et al., 2022; Joo et al., 2018; Karunatillaka et al., 2022; Mamtimin et al., 2024; Yao et al., 2024). SSN analyses consistently showed PETases group into

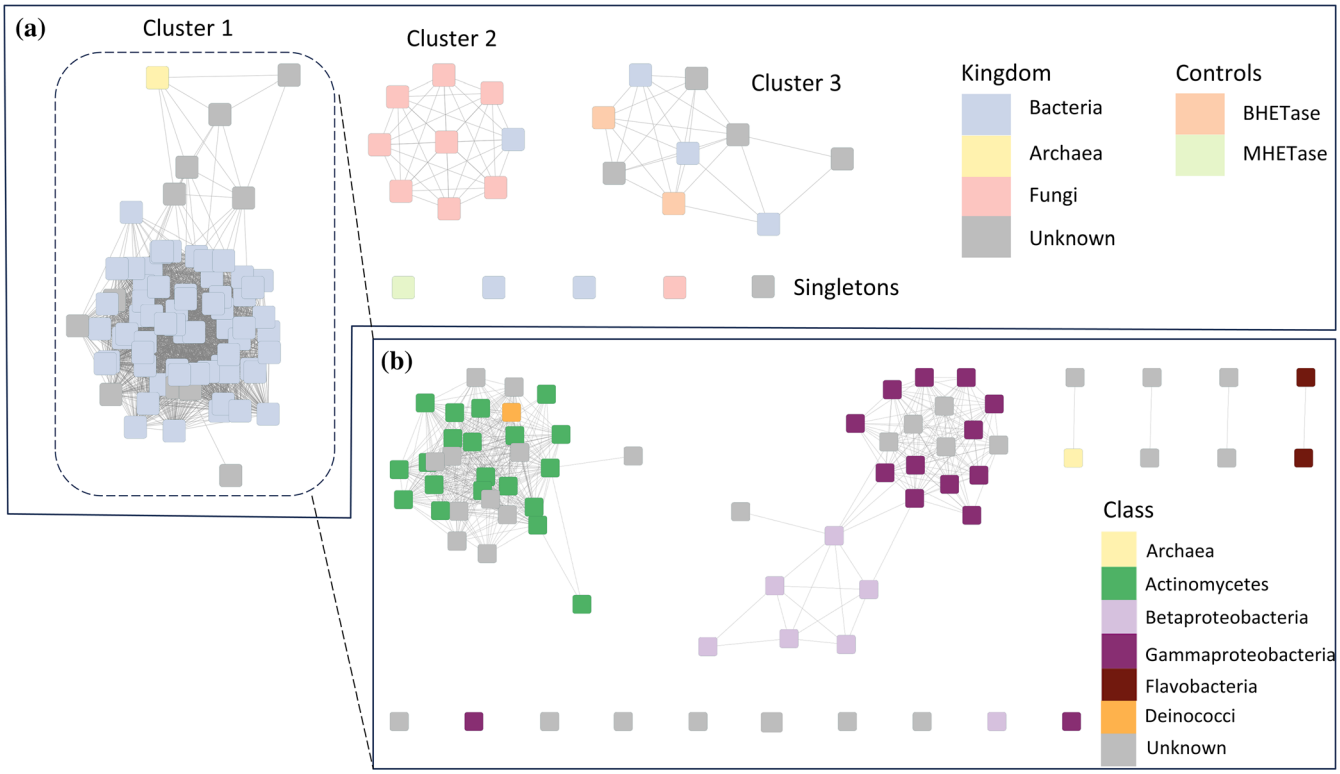

FIGURE 1 PETases sequences cluster according to taxonomic origin. (a) Low-resolution SSN (alignment score threshold = 1, ≈20% sequence identity) of 94 PETase sequences and three enzymes acting on PET degradation products: MHETase from *Ideonella sakaiensis* (Yoshida et al., 2016), and two BHETases from *Bacillus subtilis* and *Chryseobacterium* sp. *PET-29* (Li et al., 2023). Cluster 1 includes bacterial enzymes and one archaeal enzyme. Cluster 2 consists of fungal enzymes and one bacterial enzyme. Cluster 3 contains PETases tested against PET trimer (3-PET), PETases with marginal activity against PET polymer, and the two BHETases. Four PETases and the MHETase remained as singletons. (b) High-resolution SSN (alignment score threshold = 80, ≈50% sequence identity) of the enzymes in cluster 1. Here, PETases group according to taxonomic class. Distinct clusters are observed for PETases from *Actinomycetes* and PETases from *Proteobacteria*. (a) and (b) SSN analysis of PETase sequences was performed using the EFI-SSN web server (Gerlt et al., 2015) using default parameters.

3–5 clusters, with 2–8 enzymes remaining as singletons (Erickson et al., 2022; Perez-Garcia et al., 2023; Qi et al., 2023; Seo et al., 2025). Moreover, the SSN analysis by Perez-Garcia et al. (2023) further suggested that the clustering aligns with the enzymes' taxonomic origin. Enzymes within each cluster share sequence identity above a user-defined threshold. The presence of multiple clusters and singletons demonstrates that not all PETases share high sequence similarity. This may indicate that the PETase function, whether a result of positive selection or of promiscuous activity, is readily available in enzymes from different origins.

Through PANDA, these trends can be visualized and quantified. Indeed, PETases show high sequence diversity; pairwise alignments show that 54% of pairs are less than 30% identical, and 38% are less than 20% identical. Four enzymes—IS11, RmL, CbotuEstA, and LipMRD9—share less than 25% identity with any other enzyme in the dataset. The SSN of PANDA at the lowest sequence identity threshold (∼20%) groups them into three clusters and five singletons, which include the four enzymes mentioned above (Figure 1a). As previously shown by Perez-Garcia et al. (2023), two clusters in the SSN of PANDA align with the taxonomic kingdom: cluster 1 (74/94 enzymes, 78.7% of PANDA) includes bacterial and one archaeal PETase, and cluster 2 (9/94, 9.6% of PANDA) includes mainly fungal PETases. The enzymes in Cluster 3 (7/94, 7.4% of PANDA), however, separate from Cluster 1 despite being of bacterial origin. The enzymes in this cluster catalyze a PET trimer (3-PET) as a substrate or exhibit marginal activity toward larger PET polymers. This suggests that taxonomic origin is not the only clustering determinant, and that SSNs could perhaps distinguish between enzymes acting on small PET molecules and enzymes degrading larger PET polymers. Further research outside the scope of this review is needed to verify these observations. Finally, we noted that enzymes in different clusters significantly differ in size: cluster 2 contains the smallest PETases (median 19.96 kDa; e.g., *Aspergillus fumigatiaffinis cutinase*, 19.6 kDa), cluster 1 contains medium-sized PETases (median 28.55 kDa; e.g., LCC, 27.9 kDa), and cluster 3 contains large PETases (median 34.3 KDa; e.g., enzyme 101, 32.1 kDa) (Supplementary Figure 1).

As most PETases belong to cluster 1, we resolved a higher-resolution SSN (∼50% sequence identity) of

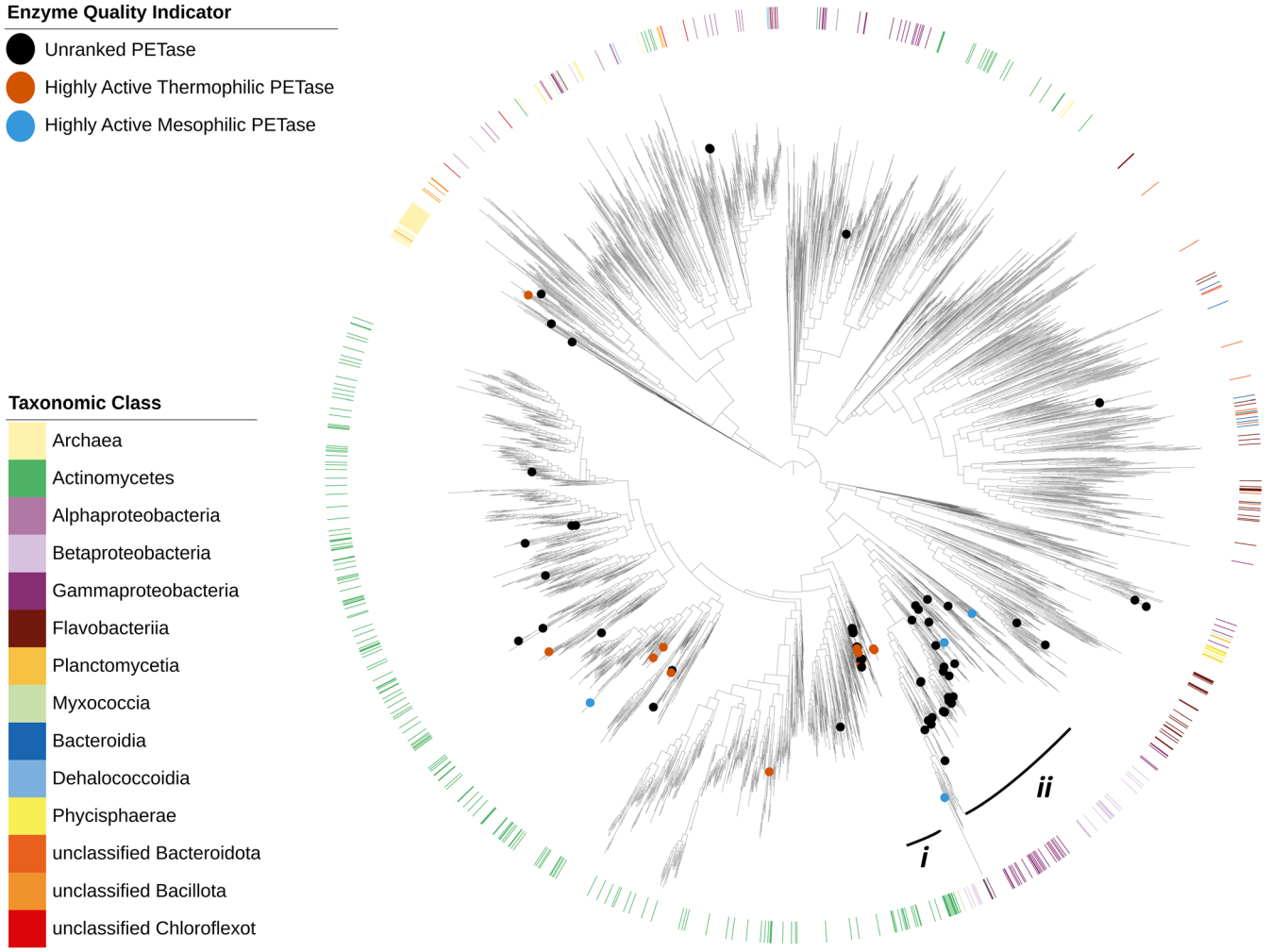

FIGURE 2 PETase activity is accessible for enzymes from multiple origins. A maximum likelihood phylogenetic tree of 4133 unique homologs of PETases from cluster 1 (Figure 1a). Homologs were retrieved by BLASTp using 40%–90% sequence identity cutoff. Duplicates were removed at a 90% cutoff using cd-hit (Fu et al., 2012) and the sequences were aligned using MAFFT (Katoh et al., 2002). The dots represent PETase query sequences, with colored dots indicating their activity ranking (see Section 4). The colored strip denotes the taxonomic class of the source organism when available. Clades i and ii are enriched with PETases. The tree was calculated using IQ-tree (Minh et al., 2020) and visualized and rooted on the mid-point using iTOL (Letunic & Bork, 2021).

this cluster. Two distinct sub-clusters, 10 singletons, and four pairs can be observed (Figure 1b). Here, clustering aligns with taxonomic class; the two largest sub-clusters belong to Proteobacteria and Actinomycetes, respectively. Interestingly and as previously shown (Joo et al., 2018; Meyer Cifuentes et al., 2022; Zhang et al., 2022), the taxonomy-based clustering correlates with structural features described by Joo et al. (2018) and Duan et al. (2023); Type I PETases with one disulfide bond and shorter loops are found in the actinomycetes sub-cluster, while Type II PETases from the proteobacteria sub-cluster feature an additional disulfide bond and extended loops.

Similarly to SSNs, phylogenetic analyses of PETases and related sequences have shown that PETases are spread across different clades, aligning to some extent with their taxonomic origin (Erickson et al., 2022; Joo et al., 2018; Karunatillaka et al., 2022; Mamtimin et al., 2024; Yao et al., 2024). To verify and generalize these results, we searched for PETase homologs using all sequences from clusters 1 and 2 (Figure 1a) as queries and performed phylogenetic analyses. In both the bacterial and archaeal tree (Figure 2) and the fungal and actinomycetes tree (Supplementary Figure 2), PETases are spread across multiple clades. However, specific clades are more enriched with PETases. In the fungal tree, a single clade within the *Sordariomycetes* class contains most cluster 2 PETases (6/9, 67.7%). In the bacterial tree, two clades belonging to the *Actinomycetes* and *Proteobacteria* phyla (marked i and ii, respectively, in Figure 2) are significantly enriched with PETases, collectively containing 50 of the 74 PETases from cluster 1. Clade i contains TfCut2 from *Thermobifida fusca*, the

first PETase to be discovered (Müller et al., 2005), and clade ii contains IsPETase from *Ideonella sakaiensis* (Yoshida et al., 2016), which received wide attention as the first enzyme whose PET degradation capabilities were shown to serve a physiological role. The fact that many novel PETases were found using sequence similarity to TfCut2 and IsPETase could account for this enrichment (Danso et al., 2018; Eiamthong et al., 2022; Erickson et al., 2022; Han et al., 2024; Hong et al., 2023; Qi et al., 2023; Ribitsch, Herrero Acero, Greimel, Dellacher, et al., 2012; Ribitsch, Herrero Acero, Greimel, Eiteljoerg, et al., 2012; Sagong et al., 2021; Wei et al., 2014). Because (a) structure-based searches (Wu et al., 2024), (b) sequence-based searches using less studied PETase (Brinch-Pedersen et al., 2024), and (c) environmental sampling (Distaso et al., 2023; Perz et al., 2016; Sulaiman et al., 2012) have been conducted, 44 out of 94 PETases (∼47% of the PETases in PANDA) are not found in these primary clades, showing that the PETase function is available for enzymes from different origins. Whether the capacity to degrade PET has physiological relevance remains unknown for most PETases, as it could be a promiscuous feat due to the chemical similarity between PET and natural polyesters. Finally, the SSN and phylogenetic trees of PETases do not match perfectly the tree of life. For example, one bacterial enzyme is found in the fungal cluster (Figure 1a, cluster 2), and actinobacteria and proteobacteria enzymes spread across multiple clades (Figure 2)—suggesting that horizontal gene transfer has played a role in the evolution of this enzyme family.

3 | THE STRUCTURE OF PETases IS HIGHLY CONSERVED: THE OPTIMIZATION STRATEGIES CONVERGE

PETases usually adopt the *α*/*β* hydrolase (ABH) fold (Billig et al., 2010; Erickson et al., 2022; Sonnendecker et al., 2022; Xi et al., 2021), the most common fold for esterases (Lenfant, Hotelier, Velluet, et al., 2013b). The only exception is the enzyme IS11, which belongs to the beta-lactamase/transpeptidase-like superfamily (Distaso et al., 2023). The ABH superfamily is a prominent protein family exhibiting sequence and functional diversity, encompassing enzymes and non-enzymes (Lenfant, Hotelier, Bourne, et al., 2013; Lenfant, Hotelier, Velluet, et al., 2013). Moreover, proteins of this fold exhibit promiscuity (Marchot & Chatonnet, 2012) and remarkable plasticity, as few mutations can cause an enzyme to change its function (Li et al., 2008; Padhi et al., 2010; Rauwerdink & Kazlauskas, 2015). PETases are no exception as they have been shown to degrade other natural and synthetic poly- and monoesters such as cutin (Kleeberg et al., 2005; Sulaiman et al., 2012), cellulose acetate (Shirke et al., 2017), polyethylene-2,5-furandicarboxylate (PEF) (Austin et al., 2018), polycaprolactone (PCL) (Sulaiman et al., 2012), and various *para*-nitrophenyl ester substrates (Yoshida et al., 2016; Zhang et al., 2024). This suggests that the capacity of some enzymes to degrade PET did not evolve by strong positive selection but is rather a result of the promiscuous activity of flexible active sites. Moreover, these observations align with PETases' high sequence diversity; natural promiscuity could explain how PETase activity emerged from different members of the ABH family.

ABHs occasionally possess additional domains like caps, lids, and binding domains (Bauer et al., 2020; Khan et al., 2017). Most PETases, however, lack these additional domains. Given the bulkiness of PET, extra domains could hinder substrate access to the active site (Khairul Anuar et al., 2022). Erickson et al. (2022) and Perez-Garcia et al. (2023) identified and discussed some exceptions.

At a systematic level, Erickson et al. (2022) identified numerous active PETases (38.4% of PANDA). They analyzed their structures, showing that most have high structural similarity to known PETases, while some exhibit truncations or extensions. An all-versus-all structural comparison of PETases from PANDA—using AlphaFold models for all enzymes—shows the same trend. As with many protein families (Higgins & Carrington, 2014; Illergård et al., 2009; Ingles-Prieto et al., 2013), the structural similarity of PETases is higher than the sequence identity (Figure 3). The conservation in structure, despite moderate sequence identity, has allowed beneficial mutations to be transposed between PETases, enhancing their activity. For example, Tournier et al. (2020) introduced a disulfide bridge from TfCut2 into LCC, resulting in LCC-ICCG. Weigert et al. (2022) successfully introduced mutations inspired by IsPETase into the wild-type PETase PET6. Fritzsche et al. (2023) and Pfaff et al. (2022) successfully introduced mutations inspired by DuraPETase, a variant of IsPETase, to the enzymes LCC-ICCG and PES-H1 (referred to as PHL7 in PANDA), respectively. Moreover, Wang et al. (2025) showed that a lysine mutation in a specific position improved the activity of six PETases. The alignment of several prominent engineered enzymes to enzyme 611 (Erickson et al., 2022), a naturally occurring cluster 1 PETase, highlights and generalizes this trend (Figure 4 and Supplementary Table 3). Indeed, many previously identified beneficial mutations could be transposed, demonstrating the practical potential of PETases' structural similarity.

4 | COMPARING PETases IS POSSIBLE DESPITE HIGHLY VARIABLE EXPERIMENTAL CONDITIONS

The PANDA dataset is unique in that it includes biochemical activity. This could enable rankings,

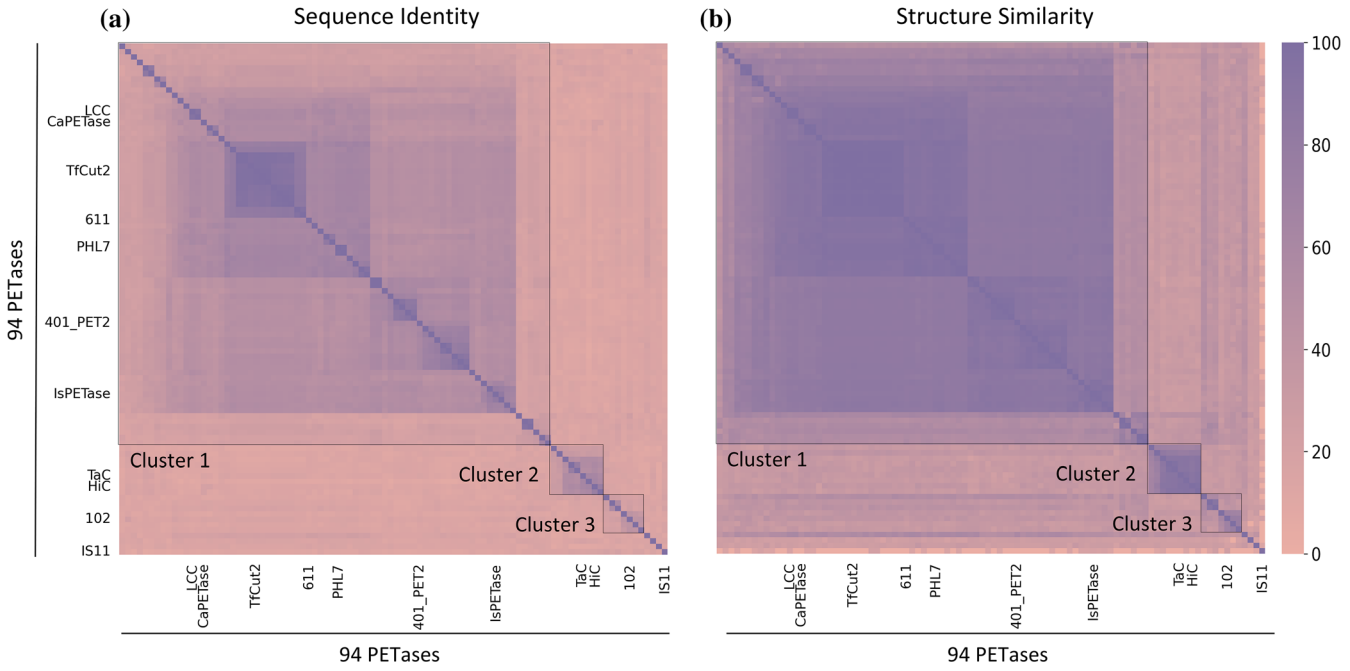

FIGURE 3 The structural similarity between PETases exceeds sequence identity while maintaining the same groupings. (a) sequence identity matrix of all PETases in PANDA, calculated using Clustal Omega (Madeira et al., 2024). (b) Structure similarity matrix based on LDDT scores (Mariani et al., 2013), calculated using Foldseek (van Kempen et al., 2024). To reduce variability, Alphafold2 predictions (Jumper et al., 2021) were used for all PETases in the dataset even when experimental structures were available. Black squares correspond to Clusters 1, 2, and 3 from the SSN analysis (Figure 1). Selected enzymes are labeled to enhance interpretability.

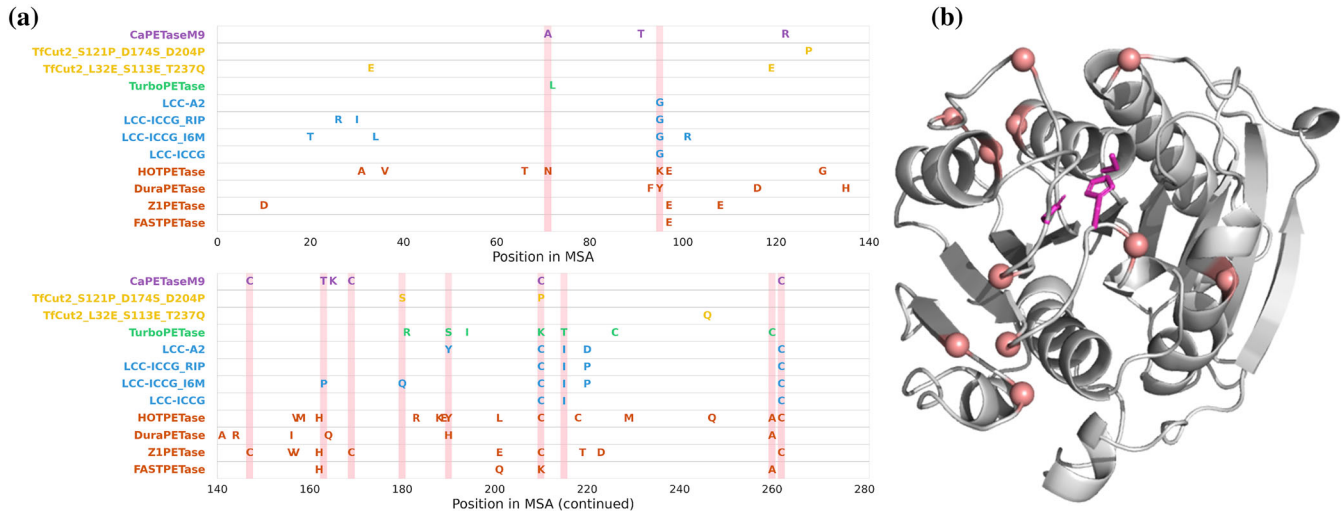

FIGURE 4 Structure-based alignment highlights highly mutable positions in PETases. (a) Structure-based sequence alignment of twelve prominent engineered PETases using FoldMason (Gilchrist et al., 2024). Positions beneficially mutated in more than one wild-type (WT) enzyme are highlighted in pink. The letter colors indicate the WT enzyme that was mutated: Purple (*CaPETase*), yellow (*TfCut2*), green (*BhrPETase*), blue (*LCC*), and red (*IsPETase*). (b) Alphafold2-predicted structure of 611, a highly active WT PETase that has not been previously engineered. Coral spheres mark positions that were beneficially mutated in multiple WT enzymes. The catalytic triad is displayed as magenta sticks. The data used to create this figure is available in Supplementary Table 3.

potentially guiding the selection of candidates for further engineering. However, PETases have been tested in highly variable experimental conditions, making direct comparisons of their activities challenging (Arnal et al., 2023; Wei et al., 2022). Although side-by-side comparisons of selected enzymes can address this issue (Arnal et al., 2023; Norton-Baker et al., 2024), this approach is impractical for the 99 enzymes identified in PANDA. To overcome this limitation, we propose using comparative rankings. For example, if enzyme A

performs better than B in one experiment and B better than C in another, it might be possible to infer that A is also better than C (A >B >C). This might not always be true; for example, a slower but more stable enzyme might outperform a faster enzyme in a long reaction but not in a shorter one (Bell et al., 2022). Substrate variability could have the same effect, as some enzymes have higher specificity toward PET film over PET powder or vice versa (Erickson et al., 2022). The extent to which such variability affects cross-experiment comparisons is still unknown.

To assess the robustness of enzyme rankings across variable experimental setups, we analyzed data by Erickson et al. (2022), where a set of 19 enzymes were tested in different conditions. We ranked the enzymes in each condition and analyzed how the rankings correlate using the Kendall Tau coefficient (Kendall, 1938). Despite the variability in substrate and reaction duration, even the lowest observed correlation (Kendall-$\tau = 0.47$) indicated a reasonable likelihood (73.5%, given by the formula $p = \frac{\tau+1}{2}$) of consistent rankings across experiments, enabling approximate comparisons of PETase activity (Supplementary Figure 3). A detailed explanation of the analysis is available in Supplementary Text 4.

To identify promising enzymes from PANDA using comparative rankings, a set of highly active enzymes serving as references must be defined. We analyzed results from another experiment by Erickson et al. (2022), where 38 PETases were tested at their optimal temperature and pH. We classified the 11 top performing as "highly active" (top ~30%). Then, we searched the rest of PANDA (61/99 enzymes) for enzymes tested against one of the 11 references when both were tested in their optimal conditions. Nine additional enzymes performed comparably or better. All 20 "highly active" enzymes (20.2% of the enzymes in the dataset) are shown in Figure 2, with their thermostability represented by color: thermophilic enzymes (optimal temperature ≥50°C) are shown in red, and mesophilic enzymes (optimal temperature ≤40°C) are shown in blue. Supplementary Table 4 contains names and thermostability classification of the highly active enzymes. The visualization of the function in the phylogenetic tree of Figure 2 shows that evolutionary origin and PETase high activity do not strictly correlate, suggesting that sequence information alone cannot be used to identify promising candidates and that highly active PETases may reside in less explored clades, as recently shown by Seo et al. (2025).

5 | ENGINEERING PETases FOR INDUSTRIAL PET RECYCLING

The first sections of this review focus on wild-type enzymes, showcasing the best naturally occurring PETases. In this section, we review the current state of PETase engineering, distinguishing between: (1) degradation in mesophilic conditions—dominated by the engineered variants of IsPETase—which provide the opportunity to integrate living cells into the recycling process, and (2) degradation in thermophilic conditions—dominated by the engineered variants of LCC—allowing PET recycling using pure enzymes and high temperatures. Detailed experimental data of the enzymes mentioned in this section are available in Supplementary Table 5.

The engineering of mesophilic PETases was boosted by the discovery of the above-mentioned IsPETase in 2016, which exhibits high specificity toward PET (Yoshida et al., 2016). The enzyme was isolated from *Ideonella sakaiensis*, a mesophilic bacterium found in a bottle recycling facility, which can utilize PET as a sole carbon and energy source. IsPETase has been extensively engineered, yielding improved variants with enhanced activity in thermophilic conditions (Bell et al., 2022; Cui et al., 2021; Joho et al., 2023, 2024; Lee et al., 2023; Lu et al., 2022; Shi et al., 2023; Son et al., 2019). Two of these, however, proved very efficient in mesophilic conditions as well. In 2020, the enzyme FAST-PETase was developed, containing five mutations compared to IsPETase (N233K, R224Q, S121E, D186H, R280A), identified using a structure-based machine learning algorithm (Lu et al., 2022). FAST-PETase produced ~6 times more degradation products compared to the wild type after 96 h of incubation at 30°C with PET amorphous film (Lu et al., 2022). Furthermore, in 2023, Lee et al. (2023) engineered Z1-PETase, containing 13 mutations compared to IsPETase—four of which are found in FAST-PETase (S121E, D186H, N246D, S242T, P181V, A180V, N37D, R132E, R224E, and disulfide bonds, N233C-S282C and A171C-S193C). Z1-PETase degraded ~75% of amorphous PET powder in 96 h at 30°C (Lee et al., 2023) and produced ~4 times more degradation products than the WT after 12 hours of incubation at 30°C with amorphous PET powder (Lee et al., 2023).

Enzymes functioning in the mesophilic temperature range (between 25 and 37°C) allow for the use of living cells as whole-cell biocatalysts, opening the door to combining multiple processes in a single bioreactor. For example, this approach would enable organisms to metabolize PET degradation monomers into valuable products without multiple reaction vessels and purification steps. This concept was exemplified by Werner et al. (2021), who engineered *Pseudomonas putida* KT2440 to produce *β*-ketoadipic acid from PET, which can then be used to produce nylon (Ackermann et al., 2024; Elmore et al., 2021). Additionally, the work of Tiso et al. (2021) demonstrated upcycling PET into two types of bioplastics.

Although FAST and Z1-PETase are suitable enzymes and the proof of concept for the production of

commodity metabolites from PET fermentation has been shown, the upcycling of PET in mesophilic conditions faces two significant challenges: First, scaling up the production of valuable secondary metabolites from the PET monomers, such as β-ketoadipic acid, is crucial for developing a process that is both efficient and viable for industrial applications (Crater & Lievense, 2018). Second, current mesophilic PETases have not yet achieved the degradation rates necessary to meet industrial demands (Blázquez-Sánchez et al., 2022).

Along these lines, another approach to enzymatic PET degradation involves using thermophilic PETases, which can function effectively at PET's glass transition temperature (Tg)—~65–75°C for amorphous bulk PET (Arnal et al., 2023; Kawai et al., 2014; Thomsen, Almdal, & Meyer, 2023). At temperatures near PET's Tg, the increased mobility of polymer chains in amorphous regions enhances degradation by facilitating enzymatic access to the ester bonds. Researchers have aimed to enhance PET degradation by engineering PET hydrolase enzymes with increased thermal stability, allowing them to remain active and efficient in elevated temperatures.

One of the first thermophilic enzymes discovered is the heavily researched Leaf Branch Compost Cutinase (LCC), identified from a metagenomic sample of leaf branch compost in 2012 (Sulaiman et al., 2012). LCC has a melting temperature (Tm) of 84.7°C (Tournier et al., 2020) and can degrade 1.45 mg of PET after 24 h of incubation at 50°C (Sulaiman et al., 2012). In 2020, the engineered enzyme LCC-ICCG was developed, containing four mutations compared to LCC (F243I, D238C, S283C, Y127G), which raised its Tm to 90.9°C. LCC-ICCG degraded 86% of amorphized postconsumer PET powder after 24 h of incubation at 72°C in a bioreactor experiment, compared to 55% by WT LCC in the same conditions (Tournier et al., 2020). In 2024, LCC-ICCG was redesigned using three rounds of protein engineering assisted by molecular dynamics analysis, resulting in the creation of LCC-A2, which contains two mutations compared to LCC-ICCG (H218Y and N248D). This enzyme has a Tm of 95.25°C and can degrade more than 90% of pretreated postconsumer PET in 3.3 h at 78°C. LCC-A2 created 1.4 times more degradation products than LCC-ICCG after 10 h of incubation at 72°C (Zheng et al., 2024). It has been shown to exhibit the highest activity when compared to 14 other thermophilic PETases under conditions of 70°C and pH 7.5 (Norton-Baker et al., 2024).

These engineering efforts enabled the industrial utilization of thermophilic enzymes. The company Carbios, founded in 2011, utilizes a variant of the enzyme LCC-ICCG to depolymerize pre-treated PET. In 2023, Carbios had a recycling capacity of approximately 250 kg of plastic daily (Buranyi, 2023). While celebrating Carbios as the first company to apply enzymatic degradation for PET recycling, there is still enormous potential for improvement in fully utilizing enzymatic PET degradation. For instance, challenges remain in addressing high-crystallinity PET in both mesophilic and thermophilic ranges (Thomsen, Almdal, & Meyer, 2023), improving PET degradation efficiency in the mesophilic range even with amorphous PET, and exploring the mineralization of PET through whole-cell biocatalysts. These challenges can be tackled by various strategies beyond improving the catalytic activity of a single enzyme. These include employing bacterial communities (Gao & Sun, 2021; Roberts et al., 2020), using enzyme cocktails (Maity et al., 2021), and incorporating adhesion molecules and binding proteins (Rennison et al., 2023). The integration of these strategies—better explained in the next section—holds significant promise toward a sustainable and efficient approach to PET recycling, contributing to a more sustainable future.

6 | FUTURE DIRECTIONS TO MEET THE CHALLENGES OF PET ENZYMATIC RECYCLING

Finally, after reviewing and commenting on PETase's discovery and engineering, we propose some future directions for addressing the challenges of the PET enzymatic recycling industry:

1. *Crystalline PET Degradation*: No enzyme has been identified that can effectively degrade the crystalline regions of PET polymers (Schubert et al., 2024). Addressing this challenge is critical for PET recycling without labor- and energy-intensive pretreatment processes (Uekert et al., 2022). Nature offers a promising model in the form of cellulosome complexes, where multiple enzymes and binding modules collaborate to break down the crystalline regions of cellulose (Artzi et al., 2017). Although PET is more hydrophobic than cellulose, a similar multi-enzyme approach has been suggested for PET degradation (Gulati & Sun, 2024; Yan et al., 2021). This strategy would involve combining different enzymes, each specializing in degrading the PET polymer or its short-chain constituents. For example, IsPETase was fused to MHETase using flexible glycine-serine linkers (Knott et al., 2020; Zhang et al., 2023) or other methods (Chen et al., 2022), resulting in improved hydrolysis. Fusion of PETase with binding modules that increase enzyme affinity to the PET surface, such as hydrophobins and carbohydrate binding modules, was successfully demonstrated as well in some cases (Jia et al., 2024; Li et al., 2024; Rennison et al., 2023; Ribitsch et al., 2015), while others questioned their contribution in industrially

relevant conditions (Graham et al., 2022; Rennison et al., 2024). Combining these approaches by fusing different enzymes with binding modules could have a positive effect by increasing enzyme-substrate interactions and tailoring enzyme functions. Such integrated systems may help overcome the challenges posed by PET crystallinity and significantly enhance overall degradation efficiency.

2. *The role of PETase half-life*: Enzyme kinetic stability, measured by half-life, is critical in designing enzymes for industrial applications as it lowers the enzyme amount required for the degradation process, making it more economically viable. Consequently, extending the half-life of PETases could significantly affect both the scalability and sustainability of enzymatic PET recycling, complementing the current emphasis on thermal stability. While most research has focused on enhancing thermodynamic stability (commonly assessed via melting temperature, Tm), less attention has been given to increasing enzyme half-life under actual degradation conditions. Although these two stability parameters can sometimes be related, they often stem from distinct mechanisms and do not necessarily correlate (Brissos et al., 2014; Xie et al., 2014). The importance of improving PETase half-life is exemplified in the work of Bell et al. (2022), which compared the performance of HotPETase (Tm of 82.5°C) and LCC-ICCG (Tm of 90.9°C) at 65°C. After approximately 5 h, HotPETase produced ~2.2 times more degradation product than LCC-ICCG. However, after ~24 h, LCC-ICCG matched and eventually surpassed HotPETase in degradation product yield due to the latter's shorter half-life under the experimental conditions (Bell et al., 2022). This example highlights the crucial role of enzyme half-life in PETase engineering campaigns and underscores the need to include half-life as a key parameter for optimizing industrially viable PETases.
3. *The optimal temperature for PET degradation*: As mentioned above, PETases are often engineered for greater thermostability (Bell et al., 2022; Lu et al., 2022; Norton-Baker et al., 2024; Tournier et al., 2020), with melting temperatures (Tm) reaching up to 98°C (Tournier et al., 2020). In temperatures near PET's Tg, the degradation rate is enhanced due to increased reaction kinetics and polymer chain mobility (Akram et al., 2024). However, the increased chain mobility at Tg enables the reordering and crystallization of chains in the amorphous phase—a process known as physical aging—compromising degradation efficiency (Thomsen, Almdal, et al., 2023a). One proposed solution is engineering enzymes with enhanced catalytic rates that outcompete the recrystallization rate (Arnal et al., 2023). However, as degradation occurs at the surface while recrystallization also affects the bulk, such enzymes must be remarkably fast. An alternative approach considers the plasticizing effect of water, which lowers the surface Tg of PET to 45–48°C (Akram et al., 2024; Tarazona et al., 2022). This suggests that degradation just below bulk Tg may balance chain mobility at the surface for effective degradation while minimizing bulk recrystallization (Kawai et al., 2024; Thomsen, Almdal, & Meyer, 2023; Thomsen, Schubert, et al., 2023). Prioritizing between fast degradation and complete depolymerization should guide the selection of the optimal temperature and, accordingly, the engineering of enzymes.

7 | CONCLUDING REMARKS

Systematically reviewing all known PETases, considering their sequence, structure, taxonomic source, and function, provides a comprehensive overview of wild-type PETases. PETases appear to have emerged from multiple origins while maintaining sufficient structural similarity to transfer function- or stability-enhancing mutations. Such transfers can guide PETase engineering campaigns, an essential step toward adapting wild-type PETases to industrially relevant conditions. Observing enzyme activity in the context of phylogenetic distribution reveals that highly active enzymes emerged from diverse evolutionary backgrounds. This indicates high levels of evolutionary convergence and shows that sequence-independent search methods would facilitate the discovery of novel and diverse PETases. Finally, our PANDA database offers a valuable resource for data-based applications as it is the first to incorporate function-related data. It may contribute to identifying novel PETases, uncovering key residues and motifs critical for PET degradation, and potentially guiding the design of de novo PETases.

AUTHOR CONTRIBUTIONS

Nitay Ahituv: Conceptualization; investigation; visualization; writing – review and editing; data curation; writing – original draft; formal analysis; software; methodology. **Dekel Freund:** Conceptualization; investigation; writing – review and editing; writing – original draft. **Raul Mireles:** Software; writing – review and editing. **Lianet Noda-García:** Project administration; conceptualization; investigation; writing – review and editing; supervision; resources; funding acquisition; writing – original draft; validation.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Lianet Noda-García https://orcid.org/0000-0002-0449-0700

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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