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Comparative Computational Analysis of Lid Conformation Effects on PET Binding in Fungal Cutinases

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Abstract
The enzymatic degradation of Polyethylene Terephthalate (PET) represents a promising sustainable approach to plastic waste management, with fungal cutinases emerging as particularly effective biocatalysts due to their ability to hydrolyse ester linkages in PET polymers. Among the structural factors that may influence catalytic performance, lid conformation appears to play a critical role in substrate accessibility and binding efficiency. Fungal cutinases exhibit diverse conformational states, ranging from open-lid structures that provide direct substrate access to closed-lid conformations that may regulate catalytic activity through controlled substrate binding. This computational study investigates how lid conformation affects PET substrate interactions by comparing two structurally distinct fungal cutinases: *Fo*Cut5a from *Fusarium oxysporum*, which exhibits an open-lid conformation, and *Tr* cutinase from *Trichoderma reesei,* characterized by a closed-lid structure. Through integrated sequence alignment, structural analysis, and molecular docking approaches, we examined the relationship between conformational state and PET binding capability. Sequence analysis revealed 39.1% similarity between the enzymes, with both containing the essential Ser-His-Asp catalytic triad and the GYSQG motif characteristic of fungal PET-degrading cutinases. While structural superimposition confirmed conserved active site architecture, the enzymes displayed markedly different substrate accessibility profiles. Molecular docking analysis demonstrated comparable binding affinities among all tested cutinases (*Fsp* cutinase: -5.27 kcal/mol, *Fo*Cut5a: -5.113 kcal/mol, *Tr* cutinase: -5.068 kcal/mol). However, significant differences emerged in substrate binding orientation: open-lid conformations facilitated proper positioning of PET ligands at catalytic serine residues, whereas the closed-lid structure resulted in alternative binding modes to non-catalytic sites. Additionally, a flexible hinge region was identified in *Tr* cutinase that may regulate lid dynamics and substrate access. These findings suggest that lid conformation represents a key structural determinant of substrate accessibility in fungal cutinases, though experimental validation is necessary to confirm the biological significance of these computational predictions. The structural insights gained provide a foundation for rational enzyme design strategies aimed at optimizing PET degradation capabilities through targeted modifications of lid architecture and dynamics.
Keywords: cutinase; PET; open-lid; closed-lid; bioinformatics tools

Introduction

Plastic is a type of synthetic polymer which is man-made from monomers derived from oil or gas through the process of polymerization. Although plastic can be broken down, it is not biodegradable. It can take up to hundreds or thousands of years to degrade (Berry et al., 2023). With the evolution of industrial advancement starting in the 1940s, the plastics industry was unlocked by the manufacturing technologies of the materials. According to the World Bank Group report, plastic comprises about 5 to 12% of the total waste generated worldwide, with 20 to 30% by weight. Approximately 60% of plastics enter the environment as plastic waste. The plastic waste generated had reached more than 360 million

tonnes (MT) in 2018 from 0.35 MT annual production in 1950, with an 8.4% annual growth rate (Kibria et al., 2023).

Polyethylene terephthalate (PET) is a thermoplastic polymer categorized in the polyester family due to the ester functionalities within its molecules. In recent years, the demand for using PET has continuously increased and has become the third most commonly used plastic in the packaging industry (Nisticò, 2020). PET waste can be disposed of in various ways, including landfills, incineration through thermal treatment, recycling, and biodegradation. Considering PET is extremely durable and may release hazardous chemicals when burning or reacting, biodegradation is the method of choice that is prioritized in the current day (Koshti et al., 2018). The enzymatic degradation is the main focus of current research because PET is composed of monomers linked by ester linkages, which can be hydrolyzed by hydrolytic enzymes such as cutinase, lipase, and PETase, which can be obtained from natural sources (Soong et al., 2022).

In addition to microbes, fungi produce cutinase and lipase, the hydrolytic enzymes that are efficient in PET degradation because they can hydrolyze the ester bonds within the monomers. Cutinase is an enzyme expressed by fungal plant pathogens when the pathogens enter the cutin. It breaks down to form the cuticle as a protective layer for the plant. Not only for PET degradation, but cutinase can also be applied in other industries. In the textile industry, cutinase can act as the catalyst in the washing of cotton fibres. The cotton that uses cutinase to wash can remove the cuticle on the cotton, resulting in better wettability (Martínez & Maicas, 2021).

In this research, fungal cutinases are the focus of work. The fungal cutinase from *Fusarium oxysporum* (*Fo*Cut5a) and *Trichoderma reesei* (*Tr* cutinase) are chosen to study due to limited studies. Sequence analysis, structural analysis, and molecular docking were performed on the chosen enzymes to gain a better understanding of PET degradation.

Materials and methods

To better understand how structural features influence PET degradation efficiency, this study employed a structured computational workflow to compare the PET-degrading capabilities of two fungal cutinases: *Fo*Cut5a from *Fusarium oxysporum* (UniProt Access Number: X0BTD8, PDB ID: 5AJH) and *Tr* cutinase from *Trichoderma reesei* (UniProt Access Number: A0A024SC78, PDB ID: 4PSC). The research design integrated both sequence alignment and structural analysis to elucidate the similarities and differences between FoCut5a and Tr cutinase, with a particular focus on active accessibility, lid conformation, and protein-ligand interaction through molecular docking.

The analysis began with the first part, enzyme analysis. The amino acid sequences of *Fo*Cut5a and *Tr* cutinase in FASTA format were retrieved from UniProt and underwent a pairwise sequence alignment in EMBL-EBI. To identify the conserved motif shared between *Fo*Cut5a and *Tr* cutinase, a multiple sequence alignment was done on *Fo*Cut5a, *Tr* cutinase and other bacterial or fungal enzymes, providing the same ability to degrade PET. Moreover, the amino acid composition of *Fo*Cut5a and *Tr* cutinase were calculated in ProtParam and classified the amino acids based on the category. To gain a better understanding of FoCut5a and Tr cutinase, a secondary structure analysis was performed. The secondary structure analysis involved determining the percentage composition of secondary structure elements using the SOPMA tool, based on the enzyme sequences in FASTA format. Additionally, the 3D models of *Fo*Cut5a and *Tr* cutinase were evaluated using a Ramachandran plot generated by PROCHECK to assess the stereochemical quality and evaluate the distribution of dihedral angles or any conformational outliers. The structural superimposition and active sites identification were performed using PyMOL. For the last section, the hinge identification was done only on the closed-lid *Tr* cutinase.

The second part of this study focused on analyzing protein–ligand interactions between PET and three fungal cutinases, which are *Fsp* cutinase (PDB ID: 1CEX, control), *Fo*Cut5a (PDB ID: 5AJH) and *Tr* cutinase (PDB ID: 4PSC). The PET ligand structure was retrieved from PubChem (ID: 18721140) and converted to PDB format in Open Babel GUI. Three protein structures were obtained from PDB and prepared in DS Visualizer and AutoDock Tools, respectively. All the PDB formats were saved in PDBQT format, including the PET ligand and the three enzymes. The molecular docking was performed

in AutoDock Vina with the grid box set to cover each active site of the enzyme. Nine docking modes were generated for each complex, and binding affinities were recorded. The best-ranked mode (Mode 1) for each enzyme was visualized using PyMOL and AutoDock Vina viewer to assess ligand orientation, hydrogen bonding, and interaction with the catalytic triad.

Results and discussion

*Fo*Cut5a and *Tr* cutinase showed a 39.1% similarity based on the pairwise sequence alignment, indicating moderate evolutionary relatedness. While the sequence identity was 26.2%, this level of similarity suggests potential homology between the enzymes. A conserved region motif with GYSQG was identified and shared between *Fo*Cut5a and *Tr* cutinase (Figure 1).

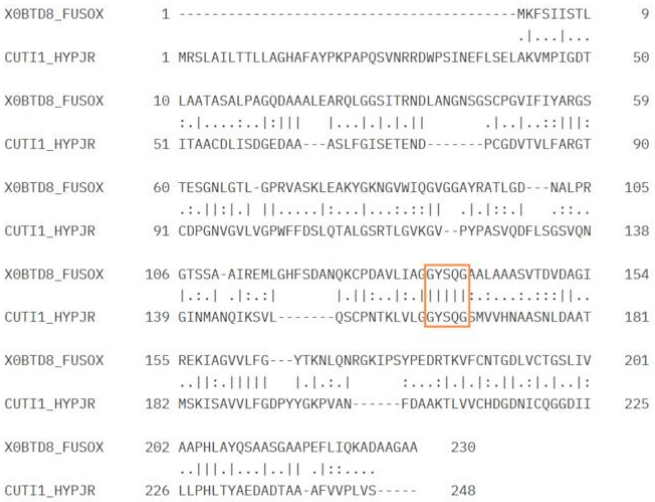

Figure 1 Pairwise sequence alignment of *Fo*Cut5a with PDB ID: 5AJH and *Tr* cutinase with PDB ID: 4PSC generated using EMBL-EBI. The orange box indicates a conserved motif shared between *Fo*Cut5a and *Tr* cutinase.

To better understand the functional significance of the conserved motif, multiple sequence alignment was performed on 19 enzymes, including two bacterial and 15 fungal enzymes with known PET-degrading capabilities. The analysis revealed the characteristic GXSXG motif commonly found in PET-degrading enzymes, where the variable positions show taxonomic specificity: fungal enzymes typically display GYSQG while bacterial enzymes show GWSMG or GHSMG variants (Figure 2). This motif is crucial for ester bond hydrolysis in PET polymers (H. Zhang et al., 2023). The presence of the GYSQG motif in both *Fo*Cut5a and *Tr* cutinase confirms their classification as fungal PET-degrading enzymes.

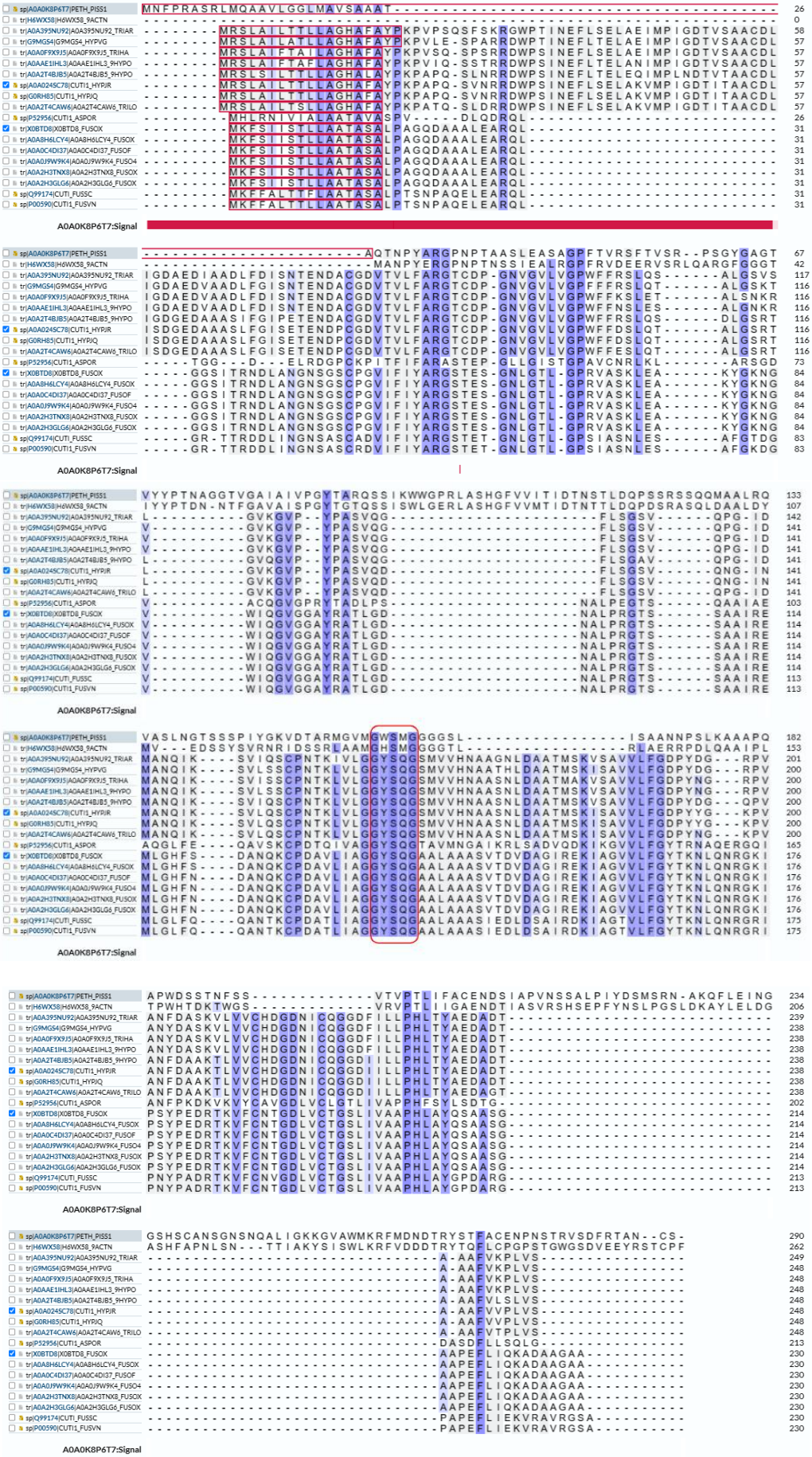

Figure 2 Multiple Sequence Alignment of *Fo*Cut5a, *Tr* cutinase and other bacterial or fungal enzymes performed using UniProt. Ticked boxes indicate Tr cutinase and FoCut5a, conserved regions are highlighted in purple and red circles the conserved motif shared among the enzymes.

The catalytic triad (Ser-His-Asp) functions to catalyze the hydrolysis of ester bonds that link PET polymers in PET plastic (Liang & Zou, 2022). *Fo*Cut5a and *Tr* cutinase both have the catalytic triad but are located at different locations in the amino acid sequences. In the superimposition between *Fo*Cut5a and *Tr* cutinase, three of the active sites for each enzyme overlapped and were aligned together (Figure 3). The same alignment of the active sites indicates that they have similar catalytic reactions. The proximity of the amino acids in the active site for *Fo*Cut5a and *Tr* cutinase was due to the precise three-dimensional alignment of the amino acid sequences. The structural flexibility of the active site offers high catalytic efficiency to *Fo*Cut5a and *Tr* cutinase, which may help improve their binding affinity with the PET ligand (Saikia & Ramakrishnan, 2022). In the view of surface representation, the aspartate active site of *Fo*Cut5a was exposed on the surface, as the hydrophobic lid did not cover it, allowing the PET substrate to interact easily. The lid of *Tr* cutinase was seen at the outside, but all the active sites were covered (Figure 4).

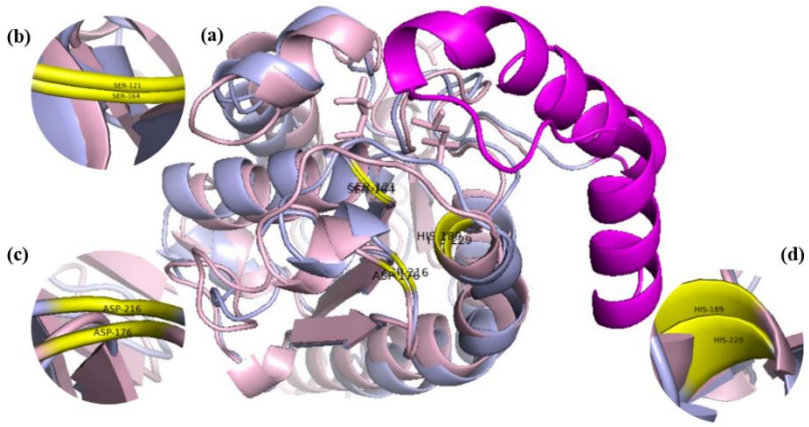

Figure 3 Superimposition of *Fo*Cut5a and *Tr* cutinase: (a) ribbon Representation of *Fo*Cut5a (pink) and *Tr* cutinase (purple), (b) serine active site, (c) aspartate active site and (d) histidine active site.

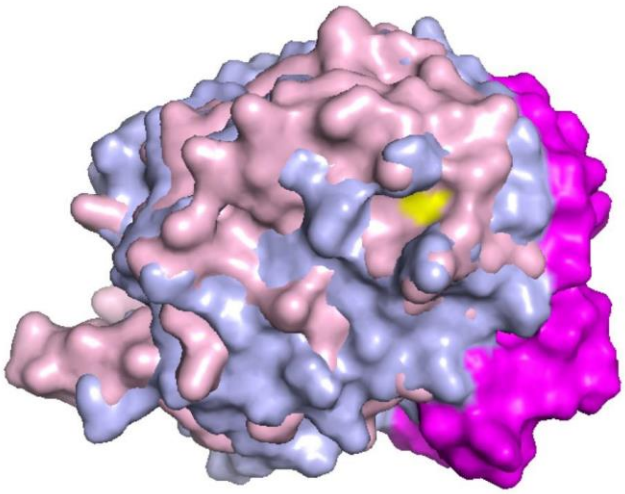

Figure 4 Surface representation of *Fo*Cut5a (pink) and *Tr* cutinase (purple) with only the aspartate active site from *Fo*Cut5a shown.

Table 1: Amino Acid Content in *Fo*Cut5a and *Tr* cutinase calculated using ProtParam

Fungal Cutinases	***Fo*Cut5a**		***Tr* cutinase**	
Amino Acid Group	**Number**	**Percentage (%)**	**Number**	**Percentage (%)**
(A) Flexible				
Alanine (A) Ala	**40**	**17.4**	**28**	**11.3**
Serine (S) Ser	18	7.8	20	8.1
Threonine (T) Thr	12	5.2	14	5.6
Glycine (G) Gly	29	12.6	22	8.9
Proline (P) Pro	9	3.9	15	6.0
Total	**108**	**46.9**	**99**	**39.9**
(B) Charged				
Lysine (K) Lys	10	4.3	8	3.2
Arginine (R) Arg	10	4.3	5	2.0
Histidine (H) His	2	0.9	4	1.6
Aspartate (D) Asp	10	4.3	18	7.3
Glutamate (E) Glu	7	3.0	6	2.4
Total	39	16.8	41	16.5
(C) Aromatic				
Phenylalanine (F) Phe	6	2.6	10	4.0
Tryptophan (W) Trp	1	0.4	2	0.8
Tyrosine (Y) Tyr	7	3.0	6	2.4
Total	14	6.0	18	7.2
(D) Aliphatic				
Cysteine (C) Cys	4	1.7	6	2.4
Valine (V) Val	**12**	**5.2**	**23**	**9.3**
Leucine (L) Leu	20	8.7	24	9.7
Isoleucine (I) Ile	13	5.7	12	4.8
Methionine (M) Met	2	0.9	5	2.0
Total	**51**	**22.2**	**70**	**28.2**
(E) Unclassification				
Asparagine (N) Asn	10	4.3	12	4.8
Glutamine (Q) Gln	8	3.5	8	3.2
Total	18	7.8	20	8.0

*Fo*Cut5a has a total of 230 amino acids, while *Tr* cutinase has a bigger size with 248 amino acids. Asparagine (N) and glutamine (Q) amino acids were not coloured as they are classified as polar but uncharged residues due to their side chains. The obvious difference between *Fo*Cut5a and *Tr* cutinase can be seen from the number of amino acids (Table 1). *Fo*Cut5a had a higher percentage of flexible group, while a higher percentage of aliphatic group was found in *Tr* cutinase. Zooming into the numerical difference of amino acid composition, alanine and valine had a marked difference in the number. *Fo*Cut5a had 40 alanine, while *Tr* cutinase had only 28 alanine. In contrast, *Tr* cutinase had 23 valines while *Fo*Cut5a had only 12 valines. Alanine enhances the solubility of protein and the hydrophobic interactions, even though it is small and non-polar. It is crucial in protein-ligand interactions, where it provides flexible regions for both the protein and ligand (Flores-Castañón et al., 2022). For valine, it is a branched, nonpolar side chain which has a strong hydrophobic ability. It is more rigid compared to smaller residues due to the presence of the bulky side chain. Thus, valine enhances the solubility of proteins and the stability of the hydrophobic protein core during protein-ligand interactions (Reifenberg & Zimmer, 2024). Therefore, the presence of alanine in *Fo*Cut5a allowed it to be more flexible, while the presence of valine in Tr cutinase provided the rigidity, which may hinder the PET ligand from interacting with the active sites. Analysis of amino acid composition revealed notable differences between the enzymes (Table 1). *Fo*Cut5a contains a higher proportion of flexible residues (17.4% vs 11.3%), while *Tr* cutinase has more aliphatic residues (28.2% vs 22.2%). The higher alanine content in *Fo*Cut5a (40 vs 28 residues) may contribute to increased structural flexibility, while the greater valine content in *Tr* cutinase (23 vs 12 residues) could enhance structural rigidity. However, the direct

correlation between these compositional differences and PET degradation efficiency requires experimental validation and should be interpreted cautiously.

Table 2: Summary of Secondary Structure Composition of *Fo*Cut5a and *Tr* cutinase generated from SOPMA

Secondary Structure	*Fo*Cut5a	*Tr* cutinase
Alpha helix (Hh)	27.43%	**31.89%**
Extended strand (Ee)	14.16%	13.78 %
Beta turn (Tt)	4.87%	3.54 %
Random coil (Cc)	**53.54%**	50.79 %

The secondary structure composition of *Fo*Cut5a and *Tr* cutinase is shown differently in the percentage (Table 2). A higher percentage of alpha helix was found in Tr cutinase, at 31.89%, which can provide stability for Tr cutinase to withstand higher temperatures (Flores-Castañón et al., 2022). *Fo*Cut5a had a higher percentage of random coil, which can accommodate substrate molecules over a wider range by providing flexibility to FoCut5a during conformational changes (Kumar et al., 2024). Ramachandran plot analysis showed that 91.8% of residues in *Fo*Cut5a and 94.6% in *Tr* cutinase were located in the most favoured regions, indicating good stereochemical quality and overall structural reliability for both enzyme models (Figure 5). Over 90% of the residues were in the most favoured regions, indicating that the models of FoCut5a and Tr cutinase were accurate and valid for use in the research (Goswami et al., 2024).

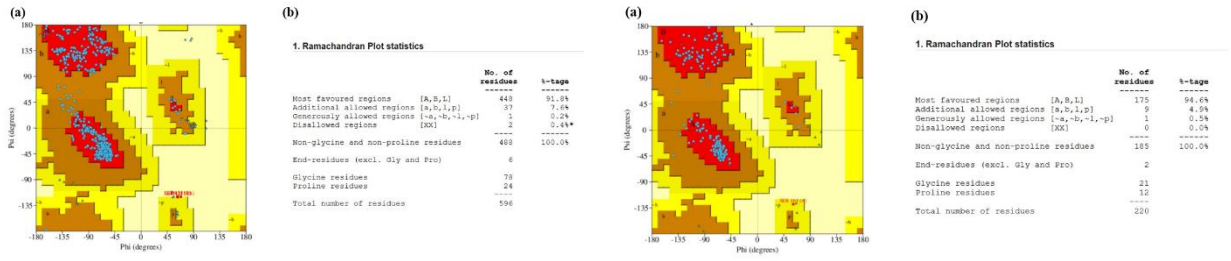

Figure 5 Ramachandran Plot of *Fo*Cut5a (left) and *Tr* cutinase (right) generated from PROCHECK

Tr cutinase contains a lid that blocks substrate access to the active site, and its movement is regulated by a hinge, which is a short, flexible amino acid sequence acting as a pivot. Using PACKMAN-Molecule, a hinge region from residues 72 to 77 was identified, which is located near the lid and composed of serine, glutamine, asparagine, threonine and aspartate (Figure 6). Although only Hinge 1 had a p-value above 0.05 (0.128), it was accepted due to its proximity to the lid.

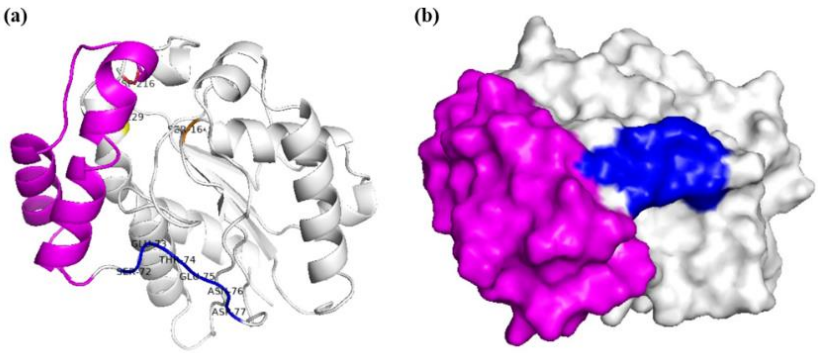

Figure 6 Representation of the lid (magenta) and hinge (blue) of *Tr* cutinase in PyMOL: (a) ribbon representation and (b) surface representation.

Table 3: Summary of Docking results from AutoDock Vina

	***Fsp* cutinase**	***Fo*Cut5a**	***Tr* cutinase**
Best binding affinity (kcal/mol)	-5.27	-5.113	-5.068
Bind to active site	Yes (Ser 120)	Yes (Ser 121)	No (Asp 237)

Molecular docking analysis revealed similar binding affinities among all three enzymes: *Fsp* cutinase (-5.27 kcal/mol), *Fo*Cut5a (-5.113 kcal/mol) and *Tr* cutinase (-5.068 kcal/mol) (Table 3). Importantly, the differences between these values are small (within 0.2 kcal/mol) and fall within the typical uncertainty range of computational docking predictions, suggesting that all three enzymes may have comparable binding potential for PET substrates.

However, a critical difference emerged in the binding mode analysis. Both *Fsp* cutinase and *Fo*Cut5a successfully positioned the PET ligand at their catalytic serine residues (Ser120 and Ser121, respectively), indicating proper orientation for catalytic attack. In contrast, the PET ligand bound to Asp237 in *Tr* cutinase rather than the catalytic serine (Figure 7). This deviation likely reflects the steric hindrance imposed by the closed-lid conformation observed in the crystal structure.

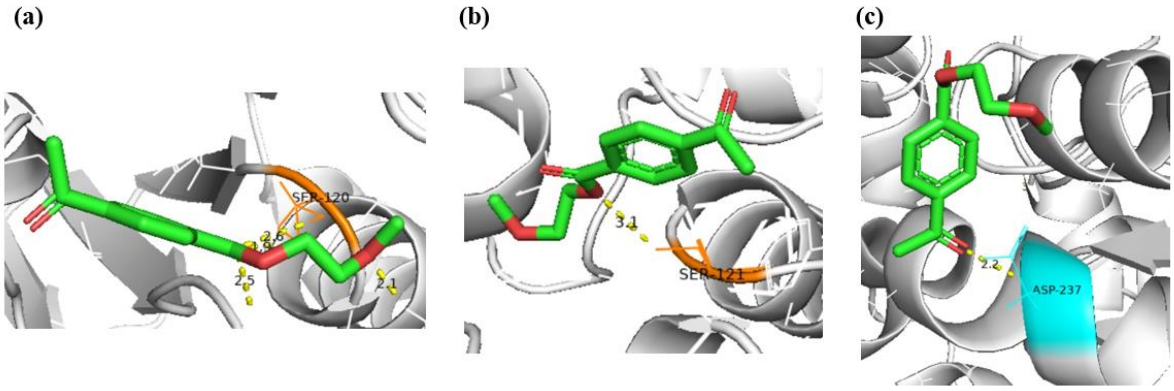

Figure 7 Binding interactions of PET ligand with (a) *Fsp* cutinase (Ser120), (b) *Fo*Cut5a (Ser121) and (c) *Tr* cutinase (Asp237). PET was correctly positioned at the catalytic serine in *Fsp* cutinase and *Fo*Cut5a, while in *Tr* cutinase, the ligand interacted incorrectly with Asp237, likely due to the closed-lid conformation. Binding distances are shown in Å.

Several important limitations should be considered when interpreting these results. First, the molecular docking employed rigid protein structures, which may not capture the dynamic conformational changes that occur during enzyme-substrate interactions. The closed-lid conformation of *Tr* cutinase observed in the crystal structure may not represent its catalytically active state, as lid opening could occur upon substrate binding through induced-fit mechanisms.

Second, the small molecule PET analogue used in docking may not accurately represent the behaviour of the actual PET polymer, which involves complex surface interactions and multiple binding events during degradation. Real PET degradation is a multi-step process that depends on factors not captured in single-molecule docking studies, including polymer chain accessibility, crystallinity, and surface topology.

Third, the computational predictions require experimental validation to confirm their biological relevance. Factors such as enzyme kinetics, thermal stability, optimal reaction conditions and actual PET degradation rates cannot be accurately predicted from structural analysis alone.

Finally, the binding affinity differences observed are within the margin of error typical for computational docking studies, and their biological significance remains uncertain without experimental confirmation.

Conclusion

This computational study provides preliminary insights into the structural features that may influence PET degradation capabilities of fungal cutinases. Sequence analysis revealed moderate similarity (39.1%) between *Fo*Cut5a and *Tr* cutinase, with both enzymes containing the characteristic GYSQG motif that defines fungal PET-degrading cutinases. The presence of conserved catalytic triads and aligned active sites suggests both enzymes possess the fundamental molecular machinery required for ester bond hydrolysis.

The most significant finding relates to the potential impact of lid conformation on substrate accessibility. While all three enzymes (*Fsp* cutinase, *Fo*Cut5a, and *Tr* cutinase) showed comparable binding affinities to PET in molecular docking analysis (differences within 0.2 kcal/mol), the binding orientations differed substantially. The open-lid conformations of *Fsp* cutinase and *Fo*Cut5a facilitated proper positioning of the PET ligand at the catalytic serine residues, whereas the closed-lid structure of *Tr* cutinase resulted in alternative binding to a non-catalytic aspartate residue.

However, several important caveats must be considered. The computational approach employed rigid protein structures that may not capture the dynamic conformational changes occurring during enzyme-substrate interactions. The closed-lid conformation observed in *Tr* cutinase's crystal structure may not represent its sole catalytically relevant state, as lid opening could potentially occur through induced-fit mechanisms upon substrate binding. Furthermore, the small PET analogue used in docking studies may not accurately reflect the complex interactions involved in actual polymer degradation, which is a multi-step process dependent on numerous factors including polymer crystallinity, surface accessibility and reaction conditions.

The binding affinity differences observed are small and within the typical uncertainty range of computational docking predictions, making their biological significance unclear without experimental validation. Additionally, the correlations suggested between amino acid composition, secondary structure content and enzymatic performance require empirical confirmation.

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