XXXII Symposium on Bioinformatics and Computer-Aided Drug Discovery (BCADD-2026)

Oral Presentation

Computational Identification of Structural Determinants associated with Transesterification Activity in Microbial Triacylglycerol Lipases

Sahana H[#], V S Vidya, and Saravanan P[*]

Department of Biotechnology & Bioinformatics

School of Life Sciences Mysuru, JSS Academy of Higher Education & Research

Karnataka, India

[#]Presenting Author: sahanah@jssuni.edu.in, *Corresponding Author: sarvananp@jssuni.edu

Introduction

Triacylglycerol lipases (EC 3.1.1.3)

- Versatile biocatalysts with diverse industrial applications.

Transesterification (TE) activity

- Enables synthesis of esters through acyl group transfer between esters and alcohols.
- Essential for biodiesel production, ester synthesis, flavor compounds, and pharmaceutical intermediates.

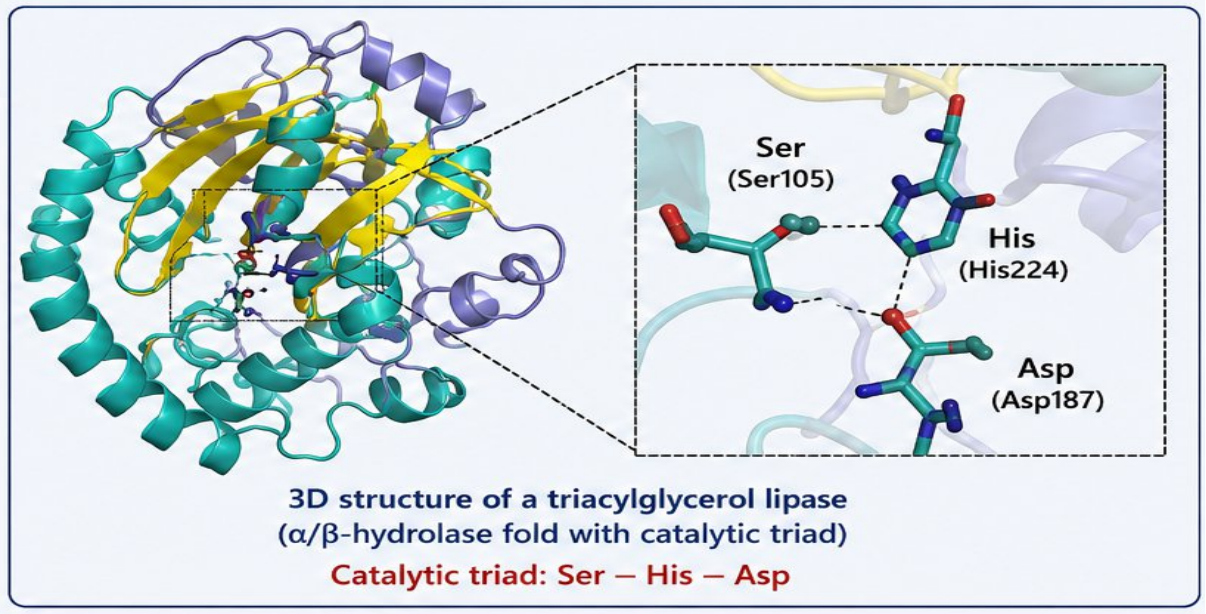
Research Gap

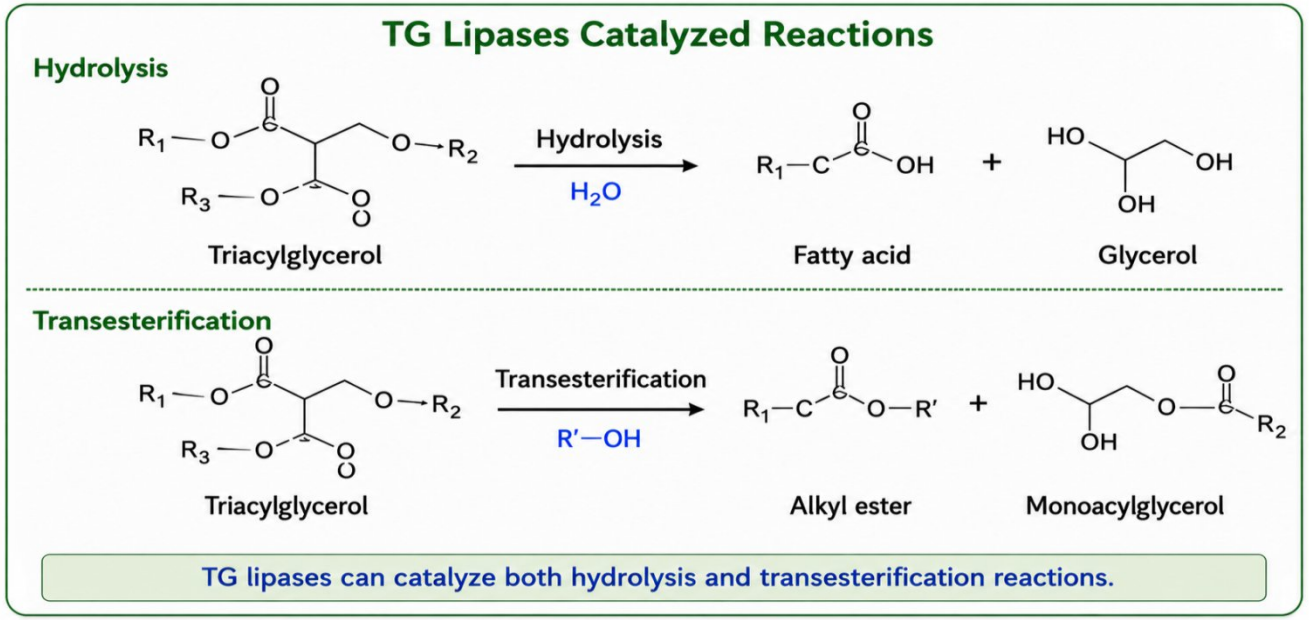
- However, only a limited number of lipases have been experimentally characterized for TE activity.
- Thousands of microbial lipases remain uncharacterized, making experimental screening expensive and time-consuming.

Role of Computational Approaches

- *In-silico* methods can rapidly analyze large datasets to identify potential transesterification- active microbial lipases.
- Identification of conserved sequence and structural determinants can guide enzyme discovery and engineering

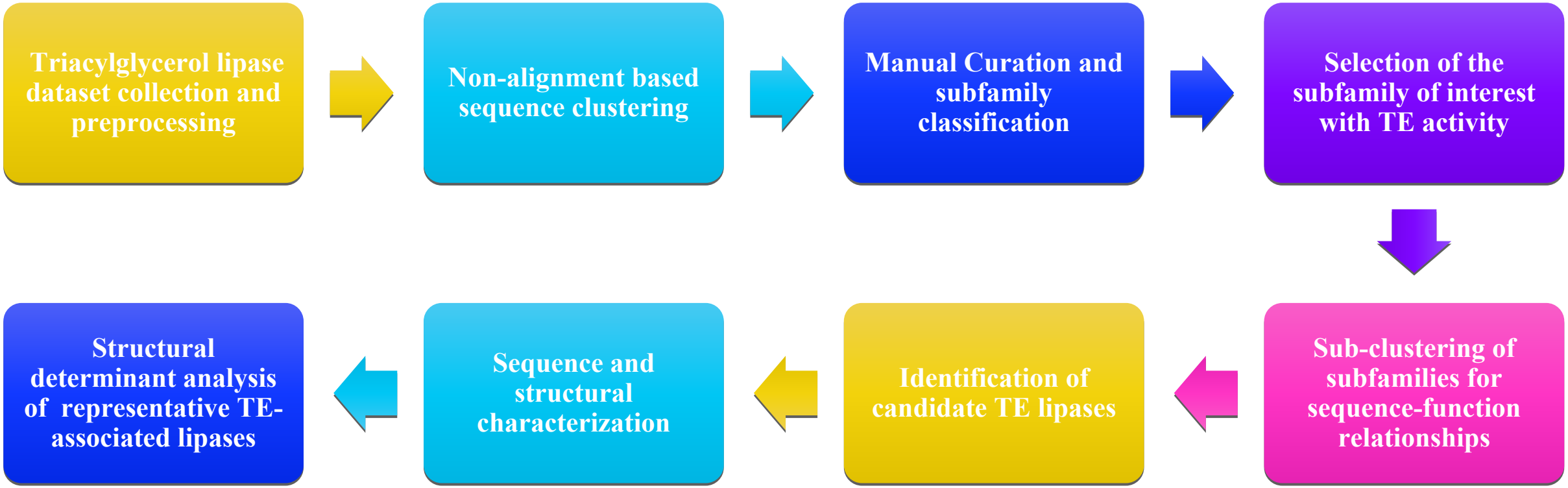
This study aims to computationally identify structural determinants associated with transesterification activity in microbial triacylglycerol lipases.





Understanding the structural determinants associated with transesterification activity can facilitate the discovery of novel microbial lipases for industrial and biotechnological applications

Methodology



Overall Approach: An integrative computational pipeline combining sequence Curation, clustering, comparative sequence analysis and structural analysis to uncover structural features that contribute to TE activity in triacylglycerol lipases

Results

Step 1: Triacylglycerol lipase dataset collection and Preprocessing

Triacylglycerol lipase sequences were retrieved from the UniProtKB database using EC: 3.1.1.3.

↓

Sequences with Protein Existence (PE) evidence were selected for further analysis.

↓

The selected PE -level sequences were preprocessed by removing redundant and fragmented sequences

↓

To retrieve and curate a high-quality, non-redundant dataset of triacylglycerol lipase sequences for downstream computational analysis

Step 2: Non-alignment-based sequence clustering

Number of clusters: 35

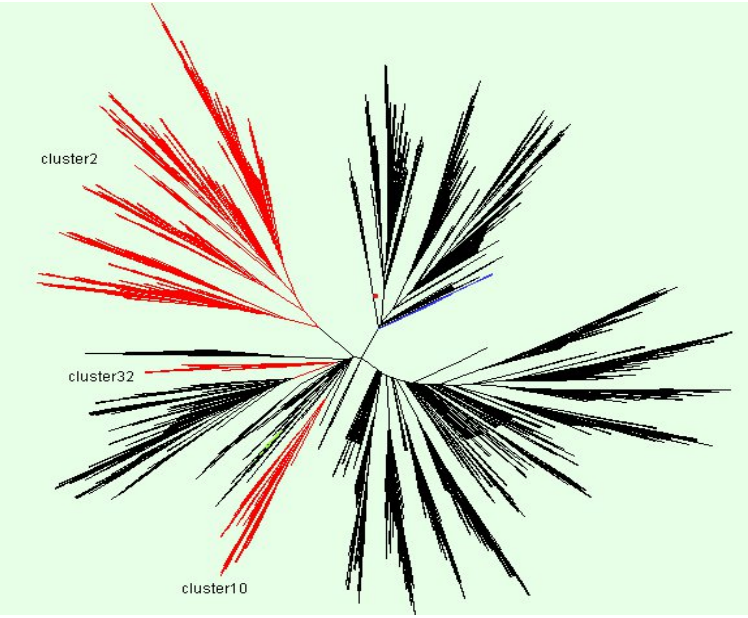


Figure 1. Dendrogram showing the hierarchical clustering of triacylglycerol lipases into sequence-based subfamilies.

Step 3: Manual curation

- Manual Curation and subfamily classification

Step 4: Secondary clustering

- Selection of the subfamily of interest with TE activity
- Sub-clustering of subfamilies for sequence -function relationships
- Identification of candidate TE Lipases

Step 5: Sub-clustering of subfamilies

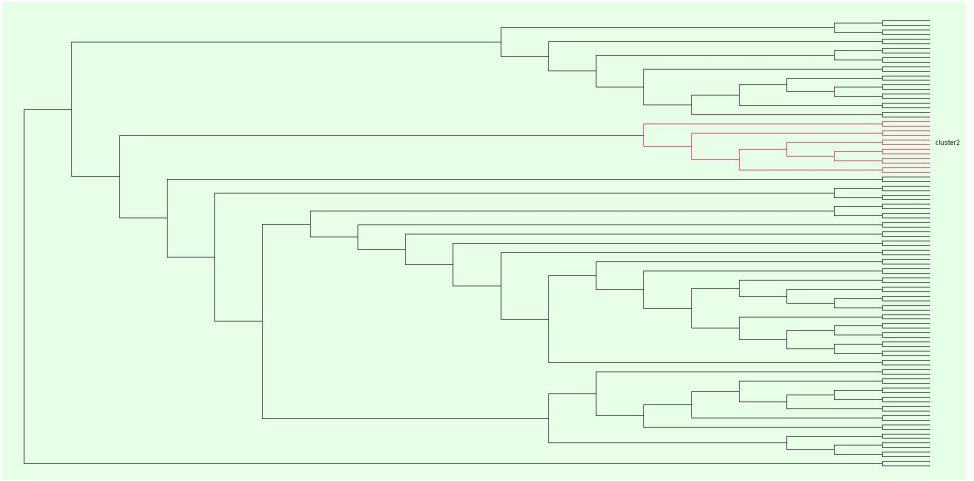


Figure 2. showing the selected subfamily containing candidate transesterification-associated lipases.

Step 6: Identification of Candidate TE Lipases & motif analysis



Figure 3. Conserved motif analysis of the selected lipase subfamily using MEME, showing conserved sequence patterns among candidate transesterification-associated lipases.

Results

Step 6: Multiple Sequence Alignment

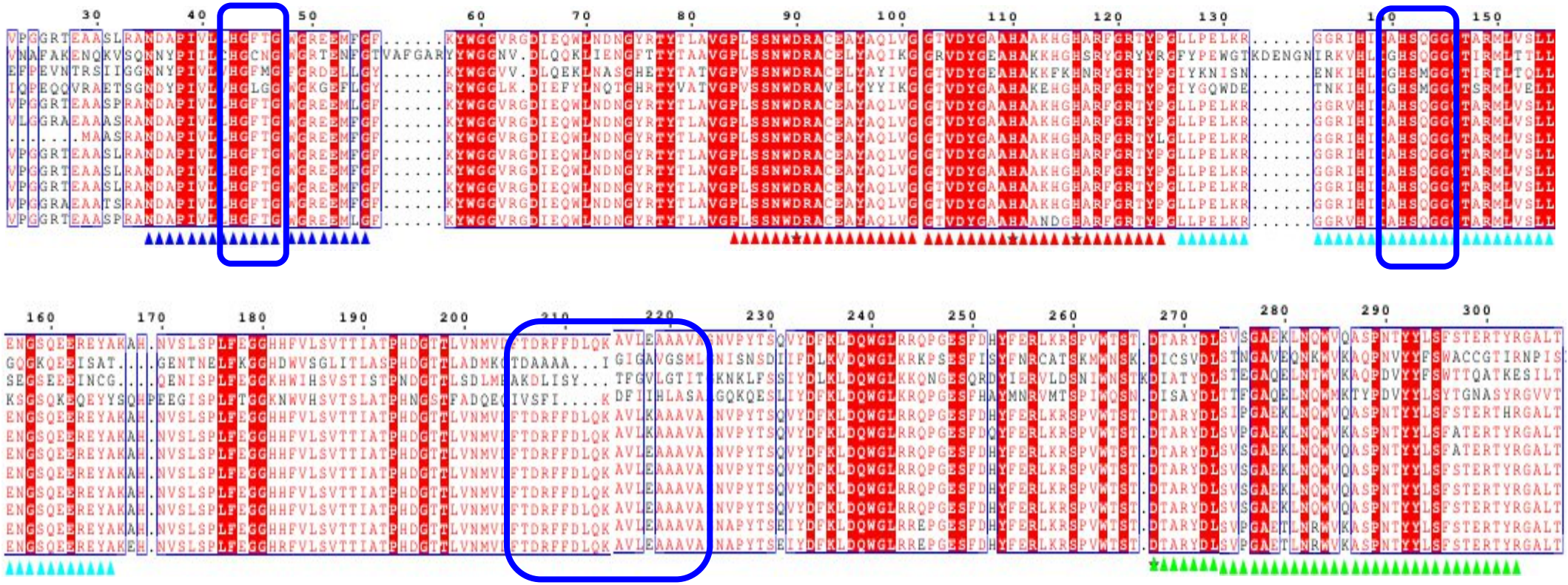


Figure 4. Multiple sequence alignment highlighting conserved catalytic residues and conserved motifs among representative candidate lipases.

Results

Step 7: Structural Analysis of Representative TE-associated Triacylglycerol Lipase

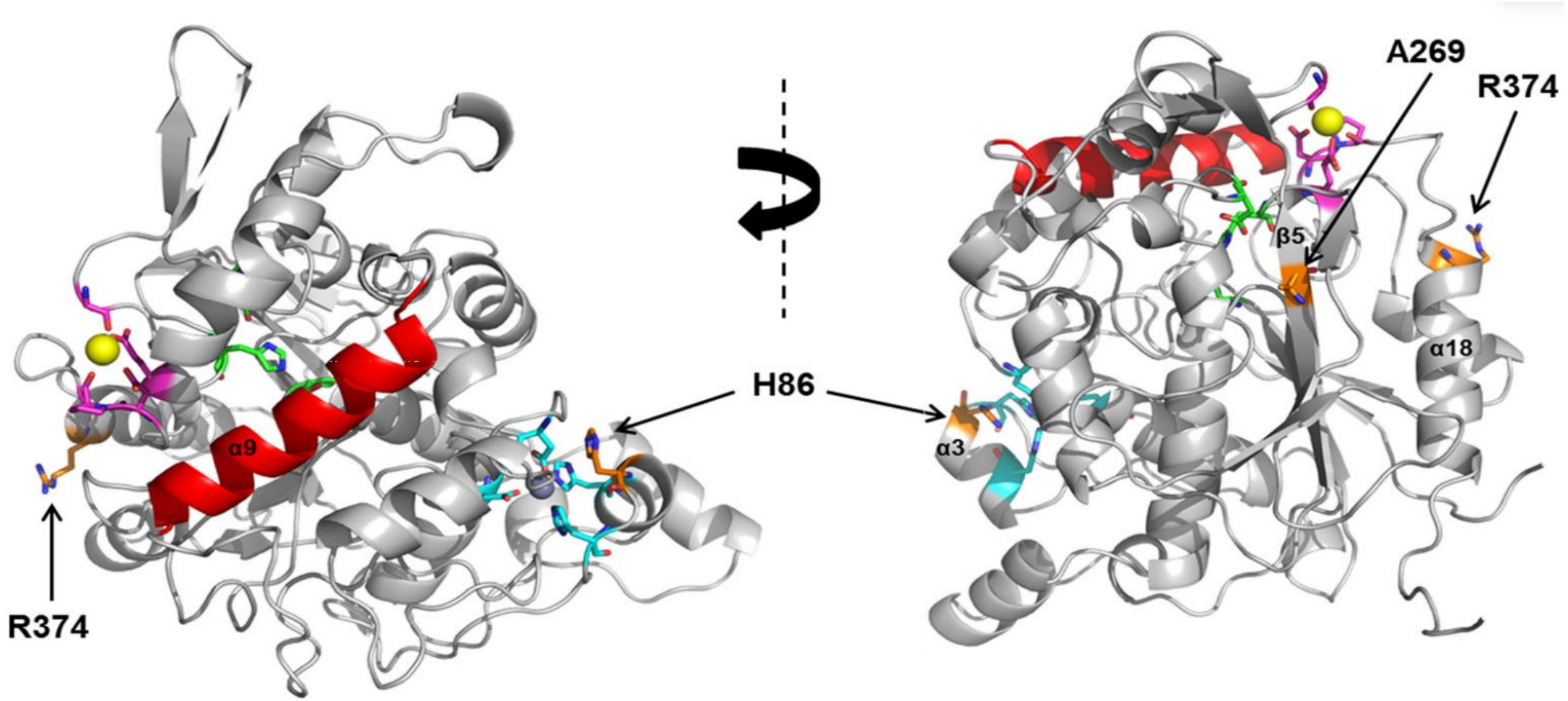


Figure 5. Crystal structure of representative Lipase T6 highlighting the catalytic triad (Ser114, Asp318, His359), lid region (Phe177–Ala192), Ca^{2+}-binding site, Zn^{2+}-binding site, and residues associated with enhanced methanol stability.

Discussion

- The computational workflow efficiently reduced thousands of microbial lipases into a small number of candidate transesterification-associated lipases through hierarchical clustering and manual curation.
- Conserved sequence motifs and active-site residues observed among the selected candidates indicate functional conservation within the identified lipase subfamily.
- Structural analysis of the representative Lipase T6 revealed conserved catalytic residues, metal-binding sites, and lid architecture that have previously been associated with enhanced methanol stability and transesterification activity.
- These observations are consistent with previous studies reporting that stabilization of hydrogen-bond networks and metal-ion binding regions contributes to improved catalytic stability in organic solvents.
- The identified candidate lipases provide a strong foundation for further computational validation and experimental characterization.

Conclusion

- Developed an integrated computational pipeline for identifying microbial lipases potentially associated with transesterification activity.
- Sequence clustering, motif analysis, and structural characterization successfully narrowed a large sequence dataset to promising candidate enzymes.
- Conserved catalytic and structural features identified in the representative lipases are consistent with characteristics reported for transesterification-active lipases.
- Limitation: Experimental validation of candidate enzymes has not yet been performed.
- Future work includes biochemical characterization of the identified candidate lipases.
- Future work will include molecular docking, molecular dynamics simulations, and experimental validation of the identified candidate lipases.
- Explore protein engineering strategies for improving catalytic efficiency and industrial applicability.

Thank you