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Structure Predictions of Hydrolase from Thermobifida Fusca Using Computational Tools

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ABSTRACT

The growing use of plastics like polyethylene terephthalate (PET) has led to increased pollution and a need for sustainable ways to break down these materials. One promising approach is using microbial enzymes through biocatalysis. This study focuses on a hydrolase enzyme from *Thermobifida Fusca (T. Fusca)*, a heat-loving bacterium known for its stable and active properties at high temperatures, making it ideal for industrial use. The main aim of this research is to predict the 3D structure of this hydrolase using homology modelling and other computational methods. The predicted structure was carefully validated using structural assessment techniques such as the Ramachandran plot to ensure high accuracy and reliability. Sequence alignment analysis helped identify evolutionary traits and potential catalytic sites, offering insights into how the enzyme functions and interacts with PET and other substrates.

Keywords: Hydrolase, plastic degradation enzyme, protein structure prediction, homology modelling

INTRODUCTION

Hydrolases are a broad class of enzymes that are of great interest in both fundamental biology and applied biotechnology. Because of their stability and high-temperature activity, hydrolases derived from *T. Fusca* are of particular interest for industrial uses such waste processing and the manufacture of biofuel. The function of hydrolase can be determined by examining its tertiary structure [1,2]. When experimental methods like X-ray crystallography and nuclear magnetic resonance are time-consuming and prohibitively expensive, homology modeling is a great alternative approach. The three-dimensional (3D) tertiary protein structure of *T. Fusca* hydrolase has not yet been precisely studied. In other words, neither experimental nor computational methods have yet to resolve this protein's 3D tertiary structure. Thus, the focus of this study is to generate the 3D tertiary structure of hydrolase from *T. Fusca* using homology modelling approach in order to provide new insights in understanding the structure-function relationship of *T. Fusca* hydrolases and their role in PET biodegradation. It highlights the environmental importance of hydrolases in tackling plastic waste. These findings support global sustainability efforts and could lead to new innovations in reducing plastic pollution and developing eco-friendly technologies.

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METHODOLOGY

The amino acid sequence of the hydrolase from *T. Fusca*, was obtained from the National Centre for Biotechnology Information's (NCBI) database. The amino acid sequence was submitted to BLAST to search for a protein template with a high similarity percentage, ideally above 30% [3]. The Clustal Omega program was employed for sequence alignment [4]. Its main objective was to align multiple sequences to identify their structural and functional relationships. It had the capability to accurately calculate and align sequences based on similarities, identities, and differences. The software MODELLER was used to generate protein models using the method of satisfaction of spatial restraints [5]. PROCHECK program was used to evaluate the quality of the model [6]. The binding sites of the tertiary model were predicted using the program Prankweb [7]. Prankweb is a computational method and machine learning specifically designed for predicting ligand binding sites in protein structures. It utilizes a graph-based approach to analyse the protein structure and identify potential binding pockets or sites.

RESULTS

A suitable template for protein *T. Fusca* was selected from the result of BLAST program which was an isomerase enzyme from *Mycobacterium tuberculosis* (PDB Id: 8H5S) with query cover and sequence identity of 92% for the former and 49.91% for the latter. A total of 100 models were generated using the MODELLER program. From there, model with the lowest energy (1125.8 kcal/mol) was selected for further analysis as it has high stability and no bumping effect [8]. The 3D tertiary structure of the selected model was generated using the VMD Visualizer as shown in Figure 1. The predicted model consisted of 42.69 % of α-helix, 9.62 % of β-strand and 47.69 % of random coil with a total of 260 amino acid residues.

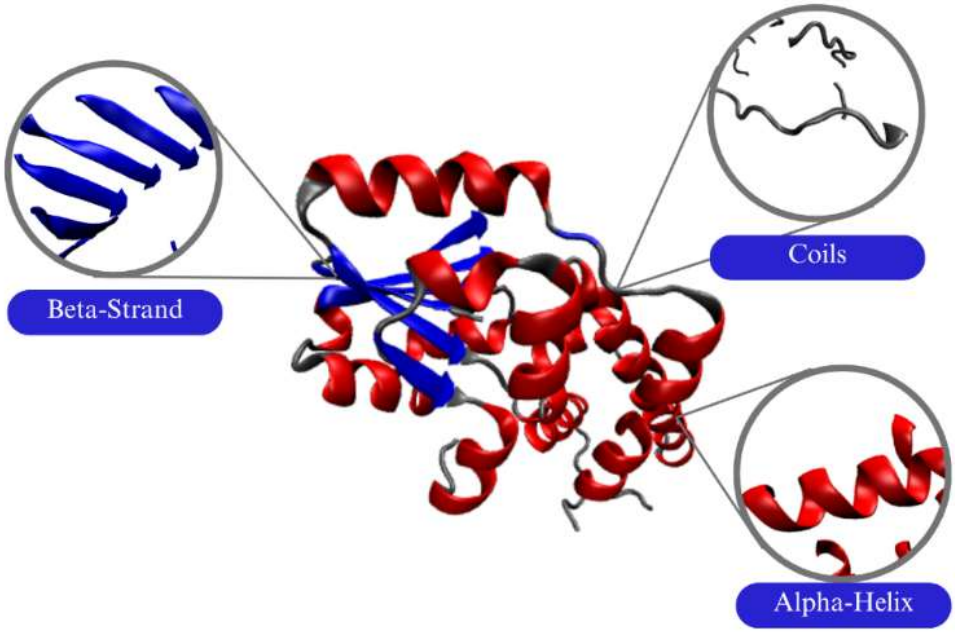

Figure 1. 3D tertiary structure model of hydrolase from *T. Fusca*

Figure 2 shows the Ramachandran Plot that obtained from the program PROCHECK. Approximately 92.5 % (196 residues) of the amino acid residues are located in the most favourable core region, which is the red region, 5.7 % (12 residues) are in the allowed region which is the yellow region. 1.9 % (4 residues) are in generously allowed region, and 0.0 % (0 residues) are in disallowed region which is the white region. Mostly, the amino acid residues were found in the core regions of Ramachandran Plot which confirms that the final structure is highly reliable for computational protein prediction methods. This result was notable because of the large percentage of residues in the preferred location (>90%) [9].

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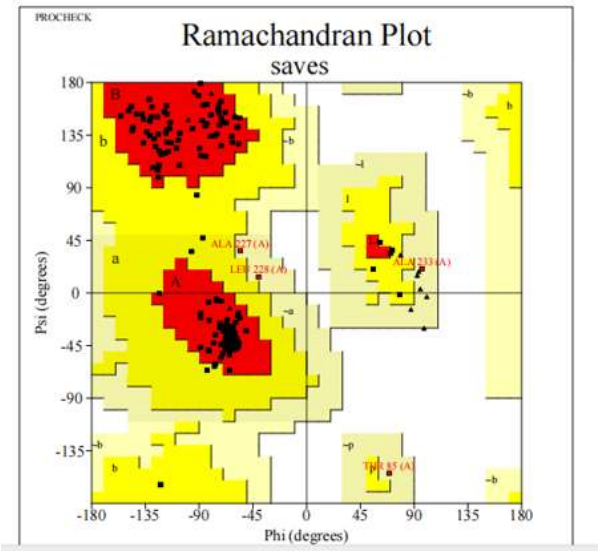

Figure 2. Ramachandran plot of tertiary structure model of hydrolase from *T. Fusca*

A protein binding site, sometimes referred to as an active site or ligand-binding site, is a particular region on a protein's surface that other molecules called ligands can bind to. To interact with ligand molecules with structural and chemical characteristics that allow them to bind specifically and firmly to a protein's binding site, these binding sites are frequently identified by their distinct form, electrostatic capabilities, and chemical characteristics [10,11]. Figure 3 shows the binding site identified using the Prankweb program. PrankWeb utilizes P2Rank, a template-free machine learning engine that maps points across a protein's solvent-accessible surface and evaluates their local physicochemical and geometric neighborhoods to predict ligandability. Surface points scoring high for potential ligand binding are subsequently clustered to define distinct binding pockets and identify the participating contact residues. From the analysis, a key binding pocket comprising seven prominent residues was identified : Lysine (LYS 103), Aspartic Acid (ASP 104), Serine (SER 140), Alanine (ALA 141), Serine (SER 142), Arginine (ARG 143), and Asparagine (ASN 144).

The predicted pocket exhibits a balanced mix of electrostatic and polar characteristics capable of driving high-affinity interactions. The presence of basic residues (LYS 103 and ARG 143) alongside an acidic residue (ASP 104) establishes a strong electrostatic field, facilitating potential salt bridge formation and charge-charge interactions with ionic ligand groups. Furthermore, the cluster of polar, uncharged residues (SER 140, SER 142, and ASN 144) provides abundant hydrogen-bond donors and acceptors essential for maintaining binding specificity. Notably, the contiguous stretch spanning SER 140 to ASN 144 suggests that a flexible loop region forms a structural border of the binding cleft, while ALA 141 offers a localized hydrophobic contact point.

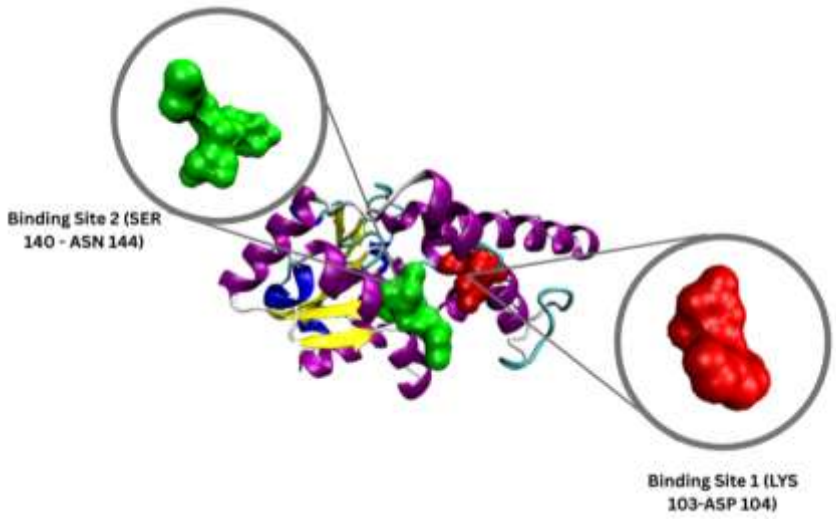

Figure 3. Predicted binding sites of the model

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CONCLUSION

The 3D tertiary structure of Hydrolase from T.fusca was successfully predicted using Homology Modelling. The binding site of the enzyme was also predicted comprises of seven prominent residues, Lysine (LYS 103), Aspartic Acid (ASP 104), Serine (SER 140), Alanine (ALA 141), Serine (SER 142), Arginine (ARG 143), and Asparagine (ASN 144). To validate these computational predictions, subsequent studies should utilize molecular docking simulations to map specific ligand-binding conformations, complemented by site-directed mutagenesis targeting LYS 103 and ARG 143 to assess their indispensable role in catalytic activity or binding affinity.

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