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Molecular Pathology and Biochemistry: Revolutionizing Diagnostic Precision in Infectious Diseases

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ABSTRACT

Background: Molecular pathology and biochemistry have significantly advanced the precision of infectious disease diagnostics, enabling rapid and accurate pathogen identification through techniques like PCR and next-generation sequencing. Homology modeling is a key computational tool in understanding protein structures, identifying drug targets, and aiding in therapeutic design.

Objective: This study aimed to perform sequence and structural analysis of the CRISPR-associated protein STY3065 from *Salmonella typhi* using homology modeling to gain insights into its structural integrity and functional roles.

Material: The genomic sequence of *Salmonella typhi* CT18 was obtained from the Comprehensive Microbial Resource (CMR). InterProScan was used to predict protein functions, and BLAST identified a homologous template from *E. coli* K-12 (PDB ID: 3NKD). Structural modeling was performed using Modeler 9.10, and the model's quality was assessed through ProCheck and ProSA.

Results: The homology model of CRISPR-associated protein STY3065 showed 94% sequence identity and 100% query coverage with the template. The model consisted of 10 alpha-helical structures and 7 beta sheets, indicating good protein flexibility. Ramachandran plot analysis showed 91.1% of residues in the most favored regions, confirming high structural quality. ProSA energy calculations indicated a stable model.

Conclusion: The study successfully generated a reliable 3D model of the CRISPR-associated protein STY3065, providing valuable structural insights. These findings highlight the potential of homology modeling in advancing our understanding of microbial proteins and aiding in the development of targeted therapies for infectious diseases.

Keywords: Molecular pathology, diagnostic precision, infectious diseases.

Introduction

Molecular pathology and biochemistry have ushered in a new era of diagnostic precision in infectious diseases, transforming traditional approaches and enhancing patient outcomes.[1] By leveraging advancements in genomics, proteomics, and metabolomics, these disciplines enable the detection of pathogens with unparalleled accuracy and speed. Molecular techniques, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), allow for the identification of microbial DNA or RNA directly from clinical samples, bypassing the limitations of conventional culture methods.[2] This rapid and specific identification not only expedites diagnosis but also guides targeted therapy, reducing the misuse of antibiotics and combating antimicrobial resistance.

Biochemical assays complement molecular tools by elucidating host-pathogen interactions and detecting biomarkers indicative of infection or disease severity.[3] Advances in mass spectrometry and bioinformatics have facilitated the discovery of novel biomarkers, enabling early detection and monitoring of infectious diseases.[4] These developments are particularly valuable in resource-limited settings, where traditional diagnostic infrastructure may be lacking. Point-of-care molecular and biochemical tests are emerging as transformative tools, delivering quick and accurate results at the patient's bedside.[5]

The integration of molecular pathology and biochemistry into clinical practice is also revolutionizing the management of infectious disease outbreaks.[6] Genomic epidemiology allows for the tracking of pathogen transmission and evolution, providing critical insights for public health interventions.[7] Additionally, personalized medicine approaches are becoming increasingly

feasible, with molecular and biochemical profiles guiding tailored treatments that consider individual patient and pathogen characteristics.[8]

Homology modeling has emerged as a transformative tool in this domain, bridging gaps in understanding pathogen structure and function.[9] By predicting the three-dimensional structures of proteins from infectious agents, homology modeling enables researchers to identify potential drug targets and design effective inhibitors.[10] This computational approach accelerates the development of vaccines and therapeutics by providing structural insights into virulence factors and antimicrobial resistance mechanisms.[11] Its application in studying transcriptional regulators of pathogens like *Staphylococcus aureus* exemplifies its potential to revolutionize infection control strategies. As a result, homology modeling is not just a powerful research tool but a cornerstone of precision medicine, paving the way for targeted, next-generation diagnostics and therapeutics in infectious disease management.[12]

Material and Methods

The precise genomic sequence of *Salmonella typhi* CT18 was obtained from the Comprehensive Microbial Resource (CMR), providing a robust foundation for in-depth genomic data analysis. This sequence enabled targeted investigations into the functional and structural aspects of the organism's proteins. InterProScan was employed to predict the functions of *Salmonella typhi* proteins. This tool analyzed the sequence to identify conserved domains, motifs, and functional sites, enabling the categorization of proteins and their potential roles in pathogenicity.

CRISPR proteins were selected for detailed structural analysis due to their critical role in adaptive immunity against foreign genetic elements. The workflow included:

The Basic Local Alignment Search Tool (BLAST) was used to identify a homologous template sequence by facilitating pairwise alignment between the target and template sequences. Structural modeling was performed using Modeler 9.10, a widely used computational tool for comparative protein modeling. Ten distinct models were generated based on the sequence-template alignment.
The most accurate and refined model was chosen based on stereochemical quality and structural integrity. ProCheck, a reliable web tool, was used to evaluate the reliability of the model by generating a Ramachandran Plot. This plot assessed the stereochemical quality of the protein structure, examining the conformational characteristics of residues across distinct regions. The energy of the CRISPR proteins was computed using ProSA (Protein Structure Analysis). ProSA facilitated the proofreading and refinement of the protein structure, ensuring that it conformed to high-quality standards and minimized structural errors.

Results

The homology modeling for a CRISPR-associated protein utilized the *E. coli* K-12 protein structure (PDB ID: 3NKD) as a template, identified through a BLAST search within the PDB database.

Description	Highest score	Over-all score	Query cover	E significance	identity	Accession
Chain A structure of CRISPR from Escherichia Coli Str.K-12 ≥PDB(3NKD)B chain B.	681	681	100%	0.0	94%	3NKDA

Figure 1: BLAST results from CRISPR (3065)

The selected template demonstrated 94% sequence identity and 100% query coverage with the target protein, STY3065. Higher identity and query coverage ensure a more accurate and reliable alignment. Modeler 9.10 was used to generate ten distinct models of the CRISPR-associated protein, and the most optimal model was selected for further analysis (Figure 2)

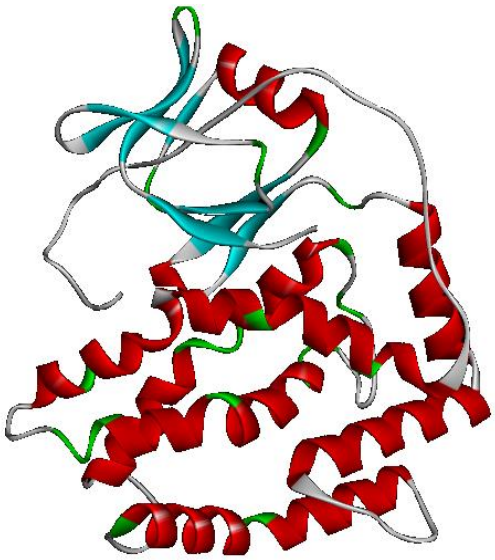


Figure- 2: Three dimensional representation of a CRISPR associated protein (3065)

The selected CRISPR-associated protein model comprises 10 helical structures and 7 beta sheets. The high percentage of alpha helices enhances protein flexibility, facilitating increased protein-protein interactions. In the structural representation, helices are depicted as red ribbons, while beta sheets are shown as blue sheets.

The Ramachandran plot, generated using ProCheck, confirmed the model's stereochemical quality. The chosen model exhibited the highest percentage of residues in the most preferred regions. Specifically, 91.1% of residues were located in the most favored region, 7.3% in additional favored regions, 1.2% in generously allowed regions, and only 0.4% in disallowed regions, as illustrated in Figure 3. This analysis underscores the structural reliability and accuracy of the chosen model.

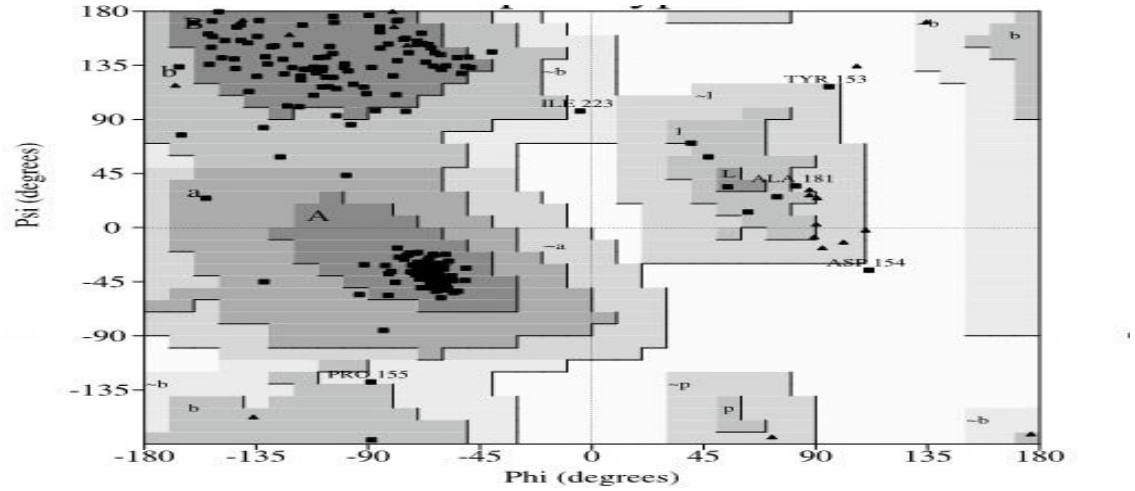
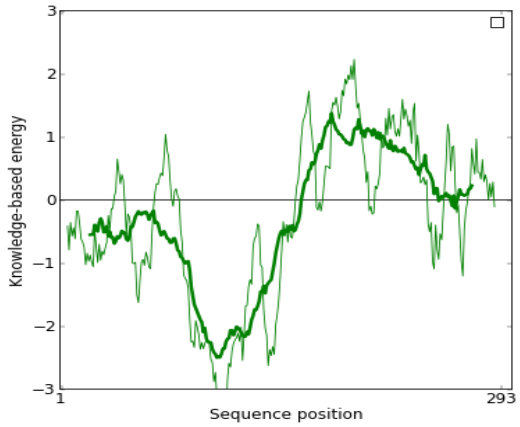


Figure -3: A plot displaying the Ramachandran angles of CRISPR associated protein (STY3065)



The energy scheme obtained from ProSA web indicated an exceptionally negative value, (Z -5.64).

Discussion

In one study, comparative alignment outcomes demonstrated that when sequence identity is 38% or higher, the quality of alignment remains consistent across various software suites.[13] However, at lower sequence identities, tools like Profit and Prime show superior performance.[14] Profit, a protein threading database, excels at generating consistent alignments for sequences with low identity by assessing their compatibility with template structures.[15]

This study focused on the sequence and structural analysis of a hypothetical CRISPR-associated protein. Our findings indicate that homology models are reliable when the sequence identity to the target structure exceeds 40%, with optimal results achieved when the sequence identity surpasses 90%. For this purpose, BLAST (Basic Local Alignment Search Tool) was used to perform precise alignments, which are essential for predicting accurate 3D structures.

Protein 3D structures provide valuable insights into the interactions and stability of proteins in their functional conformations. The template ID and query sequence were input into the $(PS)^2$ server for homology modeling using Modeler.[16] For this study, Modeler 9.10 was utilized to ensure high accuracy and reliability in the modeling process.

To assess the quality of the predicted structure, some studies rely on RMSD (Root Mean Square Deviation) for evaluating model reliability.[17] In contrast, our analysis focused on the Z-score and Ramachandran plot. The Z-score, obtained from the ProSA web tool, measures the overall quality of the model and determines if the structure falls within the expected range for native proteins of similar size. Both the query model and the template were evaluated for their Z-scores.

Additionally, Ramachandran plots were generated for both the homology model and the template to assess the quality of the phi and psi backbone dihedral angles. These evaluations confirmed that the 3D model of the CRISPR protein exhibited a reasonably accurate conformation.

Conclusion

This study successfully constructed a high-quality 3D model of the CRISPR-associated protein STY3065, demonstrating excellent sequence alignment and structural stability. The model showed favorable stereochemical quality, with a high percentage of residues in the most preferred regions of the Ramachandran plot. These findings underscore the effectiveness of homology modeling in understanding microbial protein structures and highlight its potential for guiding the development of targeted treatments in infectious disease management.

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