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Leveraging Homology Modeling for Infection Control and Regenerative Medicine: Targeting Bacterial Cell Division and Biofilm Formation

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ABSTRACT

Background: Homology modeling, which predicts protein 3D structures based on similar known proteins, is gaining traction in healthcare research, especially in infection management and regenerative medicine. In orthopedics, complications from bacterial infections like osteomyelitis and prosthetic joint infections can severely affect recovery and treatment success. This technique allows researchers to analyze bacterial proteins involved in these infections. Such insights can lead to innovative therapeutic strategies that improve patient outcomes.

Objective: To employ homology modeling to elucidate the structure and function of transcriptional regulators in *Staphylococcus aureus*, facilitating the development of novel strategies for infection control and regenerative medicine in orthopedics.

Methods: The Comprehensive Microbial Resource (CMR) is a bioinformatics tool designed for retrieving complete sequences. The Basic Local Alignment Search Tool (BLAST) was utilized for sequence alignment, while Modeller 9.10 was employed for model design. ProCheck was used to assess the stereochemical properties through the Ramachandran Plot, and the Psipred program facilitated the analysis of the secondary structure of the transcriptional regulator.

Results: Among the 10 generated models, model 3 was identified as the optimal choice. It contains 6 alpha helices and 5 beta sheets, demonstrating the highest percentage of residues located within the most favored regions of the Ramachandran Plot.

Conclusion: Innovative strategies have been developed for infection control and regenerative medicine by targeting bacterial cell division and biofilm formation. This multifaceted approach not only aims to enhance patient outcomes but also facilitates the creation of next-generation orthopedic implants resistant to infection.

Keywords: Homology modeling, biofilm, Staphylococcus aureus, transcriptional regulator, Orthopedics

INTRODUCTION

Homology modeling, a method used to predict the 3D structure of proteins based on known structures of similar proteins,[1] is rapidly advancing its role in healthcare research, particularly in infection management and regenerative medicine.[2,3] In orthopedics, bacterial infections, such as osteomyelitis and prosthetic joint infections, are major complications that can severely hinder recovery and treatment success.[4] Homology modeling can be applied to study bacterial proteins involved in these infections, providing a pathway to developing innovative therapeutic strategies.[5]

In the realm of orthopedics, the challenge of bacterial infections, particularly those caused by *Staphylococcus aureus*, presents a significant obstacle to successful surgical outcomes and patient recovery.[6] These infections often result from the formation of biofilms, which protect bacteria from both the host's immune response and antibiotic treatment.[7] To combat this growing issue, our study harnesses the power of homology modeling—an innovative computational technique used to predict the three-dimensional structures of proteins based on known structures of similar sequences. This approach is particularly useful in designing targeted interventions against bacterial cell division and biofilm formation. *Staphylococcus aureus* is notorious for its role in surgical site infections (SSIs), especially in orthopedic procedures where implants are involved.[8]The ability of this bacterium to adhere to surfaces and form

biofilms is a primary factor in its virulence.[9] Once established, these biofilms complicate treatment, as they significantly reduce the efficacy of antibiotics and facilitate chronic infections.

Bacterial cell division is a highly regulated process involving several key proteins.[10]One of the most significant targets for therapeutic intervention is the transcriptional regulator proteins that control the expression of genes essential for cell division and biofilm formation.[11]By leveraging homology modeling, we can predict the structure of these proteins in *S. aureus*, allowing us to identify potential binding sites for novel inhibitors. These proteins act as molecular switches, turning on or off the genes responsible for cell division and biofilm production.[12] Inhibiting their function could disrupt the bacterial life cycle, leading to decreased biofilm formation and enhanced susceptibility to antibiotics.[13]Using computational tools, we can create accurate models of these proteins, facilitating high-throughput screening of small molecules that can bind effectively.[14]This modeling helps elucidate the structure-function relationship, revealing how specific alterations can affect protein activity.

Biofilm formation is a complex process that involves the attachment of bacterial cells to a surface, followed by a series of coordinated events that lead to the development of a protective matrix.[15] This matrix not only shields the bacteria from the host's immune system but also from therapeutic agents. The transcriptional regulators in *S. aureus* play a crucial role in this process. They orchestrate the expression of adhesion factors and extracellular polysaccharides, which are vital for biofilm stability. By inhibiting the activity of these transcriptional regulators through designed small molecules, we aim to destabilize existing biofilms and prevent the establishment of new ones. This strategy can significantly improve treatment outcomes for orthopedic patients by reducing the incidence of SSIs.

In parallel with infection control, regenerative medicine holds great promise in orthopedics, particularly in the development of biomaterials that can promote healing while resisting bacterial colonization. Our research explores.By understanding the interactions between bacterial proteins and materials used in orthopedic implants, we can design surfaces that discourage biofilm formation and enhance tissue integration.Utilizing insights from homology models, we can develop biomimetic materials that actively engage with bacterial cells, potentially disrupting their ability to form biofilms or divide.

Objective: To employ homology modeling to elucidate the structure and function of transcriptional regulators in *Staphylococcus aureus*, facilitating the development of novel strategies for infection control and regenerative medicine in orthopedics.

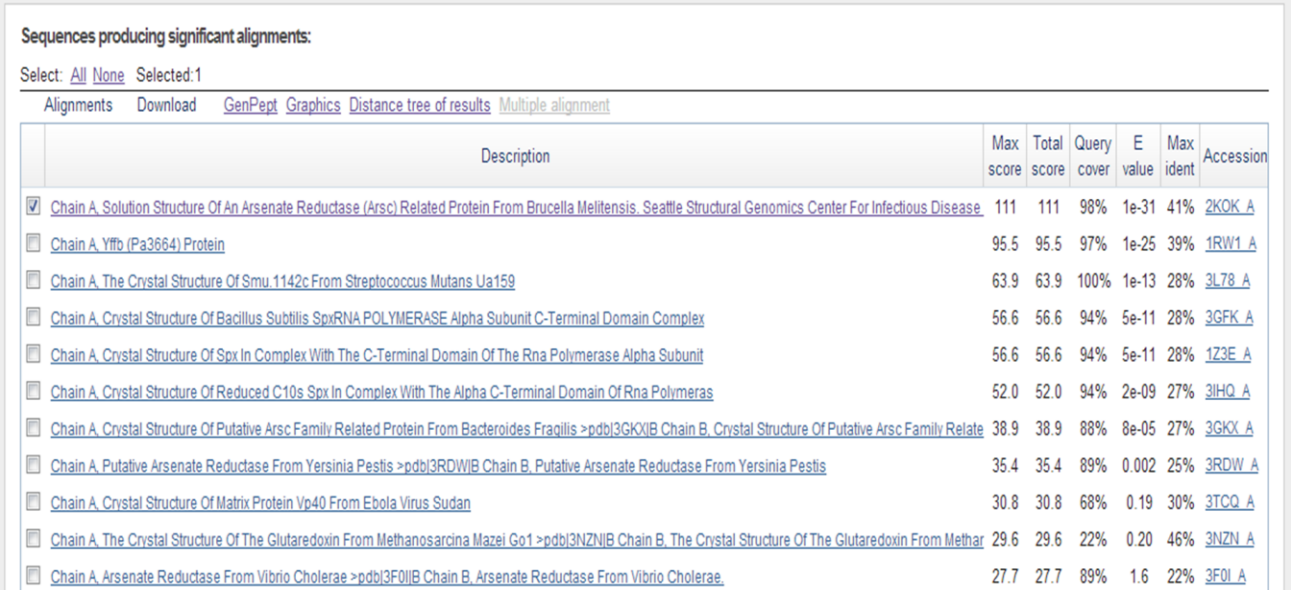
MATERIAL AND METHODS

This is a quasi-experimental study conducted under controlled conditions within a computer lab. A sample size is not necessary since there is no intent to generalize the findings to a larger population. The Comprehensive Microbial Resource (CMR) serves as an effective platform for downloading the entire genome sequence of *Staphylococcus aureus*. Approximately 3000 proteins were found in *Staphylococcus aureus*. A total of 456 hypothetical proteins with unknown functions were identified. All of these hypothetical proteins were organized and saved in a Word document. The protein's three-dimensional structure was constructed using homology modeling. In the model-building process, the template is crucial. It was retrieved by searching the Protein Data Bank (PDB) with the Basic Local Alignment Search Tool (BLAST). Modeller 9.10 was employed to construct models of the target protein, utilizing the alignment with template structures derived from the template-target alignment file. In total, 10 models were created during the modeling process, using DS Viewer software. The stability and accuracy of the models were

then evaluated using ProSA and Procheck, which offer information regarding their overall quality and stereochemical integrity.

RESULTS

To model the transcriptional regulator, we used the crystal structure coordinates of an arsenate reductase-related protein from *Brucella melitensis* (PDB ID: 2KOK) as a template. Suitable templates were identified by performing a BLAST search against the PDB database. The selection of a template for modeling was based on criteria including E-value, query coverage, and sequence identity. The target and template showed a sequence similarity of 41% as shown in figure 1 and sequence alignment was done using BLAST as shown in figure 2.



Sequences producing significant alignments:

Select: All None Selected:1

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Chain A, Solution Structure Of An Arsenate Reductase (Arsc) Related Protein From Brucella Melitensis. Seattle Structural Genomics Center For Infectious Disease	111	111	98%	1e-31	41%	2KOK_A
Chain A, Yffb (Pa3664) Protein	95.5	95.5	97%	1e-25	39%	1RW1_A
Chain A, The Crystal Structure Of Smu.1142c From Streptococcus Mutans Ua159	63.9	63.9	100%	1e-13	28%	3L78_A
Chain A, Crystal Structure Of Bacillus Subtilis SpxRNA POLYMERASE Alpha Subunit C-Terminal Domain Complex	56.6	56.6	94%	5e-11	28%	3GFK_A
Chain A, Crystal Structure Of Spx In Complex With The C-Terminal Domain Of The Rna Polymerase Alpha Subunit	56.6	56.6	94%	5e-11	28%	1Z3E_A
Chain A, Crystal Structure Of Reduced C10s Spx In Complex With The Alpha C-Terminal Domain Of Rna Polymeras	52.0	52.0	94%	2e-09	27%	3IHQ_A
Chain A, Crystal Structure Of Putative Arsc Family Related Protein From Bacteroides Fragilis >pdb|3GKX|B Chain B, Crystal Structure Of Putative Arsc Family Relate	38.9	38.9	88%	8e-05	27%	3GKX_A
Chain A, Putative Arsenate Reductase From Yersinia Pestis >pdb|3RDW|B Chain B, Putative Arsenate Reductase From Yersinia Pestis	35.4	35.4	89%	0.002	25%	3RDW_A
Chain A, Crystal Structure Of Matrix Protein Vp40 From Ebola Virus Sudan	30.8	30.8	68%	0.19	30%	3TCQ_A
Chain A, The Crystal Structure Of The Glutaredoxin From Methanosarcina Mazei Go1 >pdb|3NZN|B Chain B, The Crystal Structure Of The Glutaredoxin From Methar	29.6	29.6	22%	0.20	46%	3NZN_A
Chain A, Arsenate Reductase From Vibrio Cholerae >pdb|3F0I|B Chain B, Arsenate Reductase From Vibrio Cholerae.	27.7	27.7	89%	1.6	22%	3F0I_A

Figure 1: Blast results using the crystal structure coordinates of an arsenate reductase-related protein from *Brucella melitensis* (PDB ID: 2KOK) as a template.

```
Query  2
ITVYGIKNCDTVKKALKWLADHNIEHKLHDYRVDGLDLNFLTQAETQFGWDVLVNKRSTT  61
          +T+YGIKNCDT+KKA  WL DH I++  HDY+ +GLD   L +      W+ L+N+  TT
Sbjct  7
VTIYGIKNCDTMKKARIWLEDHGIDYTFHDYKKEGLDAETLDRFLKTVPWEQLLNRAGTT  66

Query  62   WRNLDEQVKNSLDKTTALSVLAENPTLIKRPIILQDEKALIGFNEKEYQAVF  113
          +R L E V++++D  +A  ++   P+++KRP++ +D K ++GF   +Y+A F
Sbjct  67   FRKLPEDVRSNVDAASARELMLAQPSMVKRPVLERDGKLMVGFKPAQYEAYF  118
```

Figure 2: Sequence alignment of transcriptional regulator protein with *Brucella melitensis* arsenate reductase (ArsC) having *(*PDB id; 2KOK)**.**

Ten models were created using the Modeller software (version 9.10).Out of 10 models, one best model was selected as shown in figure 3.

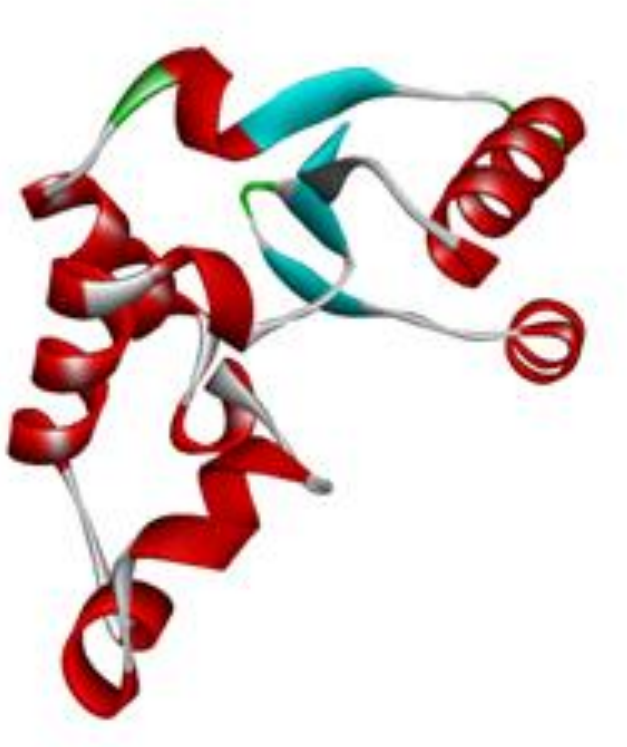


Figure 3**:** Homology model of transcriptional regulator protein of staphylococcus aureus.

The quality of the protein structures was evaluated using PROCHECK, and one model was selected based on the stereo chemical quality of the predicted protein structure.The final selected model exhibited favorable stereochemistry, with no residues falling within the disallowed region. In the Ramachandran plot, 92% of residues were located in the most favored regions, while 6.7% and 1% were found in the additional allowed and generously allowed regions, respectively (see Fig.4).

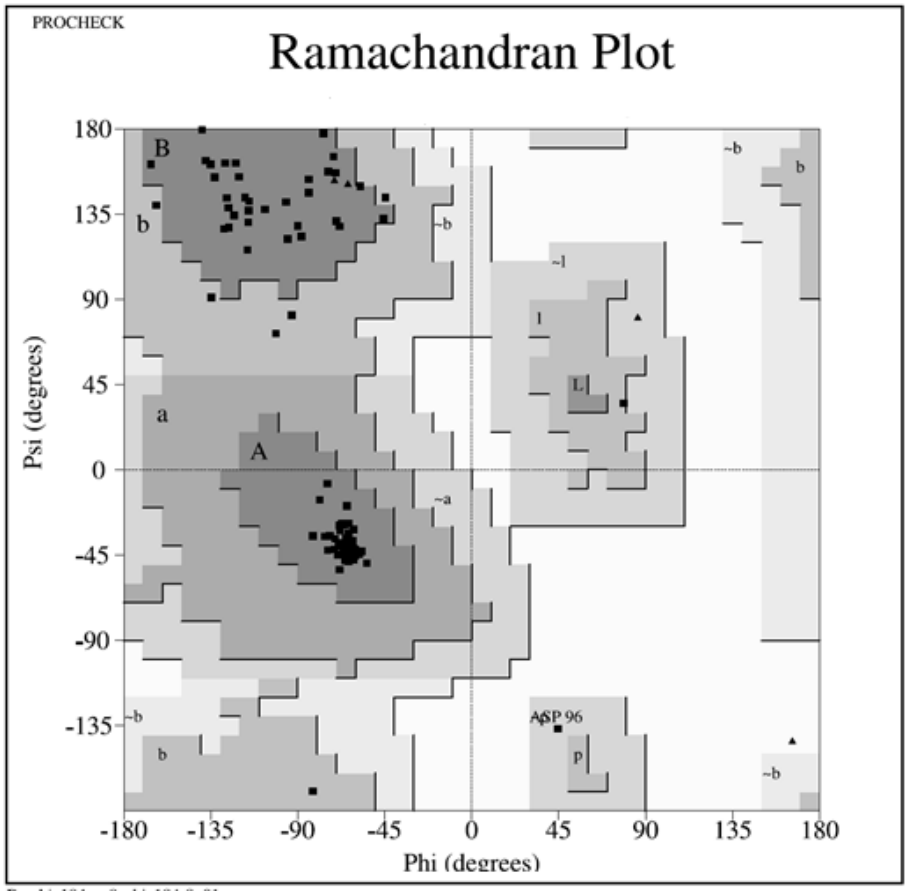


Figure-4: Stereochemical analysis of transcriptional regulator protein of staphylococcus aureus using PROCHECK.

Statical analysis from Plot:

Residues in most favored regions [A, B, L]	96	92.3%
Residues in additional allowed regions [a, b, l, p]	7	6.7%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	1	1.0%
Residues in disallowed regions	0	0

Secondary structure prediction using PSIPRED, along with Rasmol analysis, revealed that the structure consists of six helices, five beta sheets, and eight turns as shown in figure 5.

Figure-5: Secondary structure prediction of transcriptional regulator protein of staphylococcus aureus using PSIPRED.

DISCUSSION

Staphylococcus aureus is well-known for its association with surgical site infections (SSIs), particularly in orthopedic surgeries that involve implants. The present study centered on the sequence and structural analysis of a hypothetical protein transcriptional regulator of *Staphylococcus aureus* Our findings indicate that the homology models are generally deemed acceptable when the sequence identity to the target structure exceeds 40%, with optimal results achieved when this identity surpasses 90%. For this analysis, the Basic Local Alignment Search Tool (BLAST) was employed. Accurate alignment is essential for reliably predicting a consistent three-dimensional structure.

Three-dimensional protein structures provide in-depth understanding of how proteins interact and stabilize in their functional forms. The template ID and query sequence were subsequently submitted as input to the $(PS)^2$server for homology modeling using Modeler [16]. In our research, we utilized Modeller 9.10 to achieve more precise and reliable results.

In one study, RMSD (Root Mean Square Deviation) was employed to evaluate the structural reliability of the predicted model.[17] Our research focused on the Ramachandran plot, which was generated for both the homology model and the template as a quality assessment. This analysis indicated that the phi and psi

backbone dihedral angles in the three-dimensional model of the transcriptional regulator protein were reasonably accurate.

Limitations: It comes with several limitations. The accuracy of homology modeling is significantly influenced by the quality and resolution of the template protein structure. If the template is of low quality or if there is minimal sequence similarity between the template and the target, the resulting model may not be accurate. Additionally, using a homology model for further predictions without proper validation carries the risk of making incorrect conclusions regarding protein function, interactions, or drug design.

CONCLUSION

This study seeks to bridge the gap between computational biology and clinical applications in orthopedics. By leveraging homology modeling to understand the structure and function of transcriptional regulators in *Staphylococcus aureus*, we aim to develop innovative strategies for infection control and regenerative medicine. Targeting bacterial cell division and biofilm formation presents a multifaceted approach that not only promises to enhance patient outcomes but also paves the way for the development of next-generation orthopedic implants resistant to infection.

Through our findings, we hope to contribute to the growing body of knowledge that will ultimately improve surgical success rates and patient quality of life in orthopedic care.

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