

# Identification of Membrane Associated drug Targets in *Staphylococcus aureus* by Subtractive Genomics Approach

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**ABSTRACT:** In present study insilico subtractive genomics approach has been carried out to identify non homologous essential membrane proteins of *Staphylococcus aureus* Mu50. These membrane associated proteins could be effective candidates for new vaccine epitope design and for screening of potential antibacterial drugs. The essential proteins of *Staphylococcus aureus* Mu50 were identified using subtractive genomics approach and further analyzed for identification of potential drug targets.

**KEYWORDS:** Identification, Non-homologous, Membrane Proteins, New Vaccine, Epitope, Antibacterial Drugs, Subtractive Genomics.

## I. INTRODUCTION

*Staphylococcus aureus* is both a commensal bacterium and a human pathogen. Approximately 30% of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections.[1]

## II. LITERATURE SURVEY

In India, the consumption of antibiotics is considerable and the overall rate of MRSA in clinical specimens is significant. In a hospital located in eastern Uttar Pradesh, 54.8% of the *S. aureus* isolates were reported as MRSA. These isolates were also resistant to multiple antibiotics: over 80% were resistant to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, erythromycin and tetracycline.[2]. Its surface is 'decorated' with proteins that are covalently anchored to the cell wall peptidoglycan. Structural and functional analysis has identified four distinct classes of surface proteins, of which microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) are the largest class. These surface proteins have numerous functions, including adhesion to and invasion of host cells and tissues, evasion of immune responses and biofilm formation. Thus, cell wall-anchored proteins are essential virulence factors for the survival of *S. aureus* in the commensal state and during invasive infections, and targeting them with vaccines could combat *S. aureus* infections. [3]

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## III. METHODOLOGY

The complete proteome of *Staphylococcus aureus* Mu50 was retrieved from NCBI. [4] Each protein sequence of the bacteria was subjected to BLAST-P, specifically against DEG (Database of Essential Genes) available at <http://tubic.tju.edu.cn/deg/> for the screening of essential genes. [5] Cut off for E-value, bit score and percentage of identity at amino acid level were considered  $<E-10$ ,  $>100$  and  $>35\%$  respectively. Matches of length  $<100$  amino acids were not considered for further studies.

The essential proteins were subjected to human BLASTp in NCBI server. Sequences that did not show similarity with any human sequences were selected. Identification of membrane localized candidate drug targets: Sub-cellular localization of each identified non homologue essential gene was determined by Specialized Sub Cellular Localization Server P SORT. [6] Metabolic Pathway Analysis: Metabolic pathway analysis of the essential proteins of *Staphylococcus aureus* was done by KEGG Automatic Annotation Server (KAAS). [7] Comparative analysis of the metabolic pathways of the host and pathogen was performed by using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database for identification of pathogen specific metabolic pathway associated proteins. Homology modelling for 3D structure of putative drug targets: Modeling of the target proteins were performed using Swiss Modeller.[8] Energy minimization of generated 3D-model was done through SPDV (OPLS force field) by using Steepest Descent and Conjugate Gradient Algorithms. [9] Parameters like covalent bond distances and angles, stereochemical validation, atom nomenclature were validated using PROCHECK and overall quality factor of non-bonded interactions between different atoms types were measured by ERRAT program.. [10] RMSD (root-mean-square deviation) and RMSF (Root Mean Square Fluctuation) was calculated for modelled structures. Functionally important residues (Active-site) were identified through CASTp. [11] The generated 3D model of target proteins were checked by Ramachandran plot through PROCHECK program.

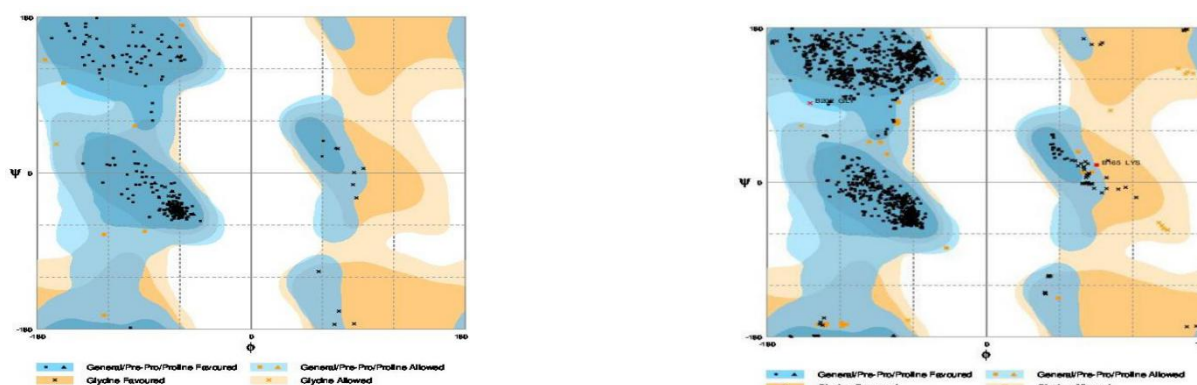
## IV. RESULTS AND DISCUSSIONS

A subtractive genomics approach utilizes the whole proteome of host and pathogen to identify proteins exclusively present in the pathogen by deducing the homologous proteins. [12] Based on the similar approach as mentioned in materials and methods, 37 essential genes in *Staphylococcus aureus* genome were found to be non homologous to human (putative drug targets). These nonhuman homologous targets identified were further analysed for sub-cellular localization prediction which detected 15 membrane localized proteins. Membrane localized proteins represent largest group (70%) of effective drug targets in any organism. [13] and can also act as potential epitopes for vaccine design.. Modelling was a tedious task due to very low sequence similarity and coverage and only five models could be obtained. Three dimensional model of drug targets were generated through identified templates along with fold fitting. Fold recognition was done through GenThreader and LOMETS server for fold assignment. Helices have dominance over other secondary structures. Ramachandran plot of the proteins analysed using PROCHECK with the description of the allowed regions are shown in the fig 1.

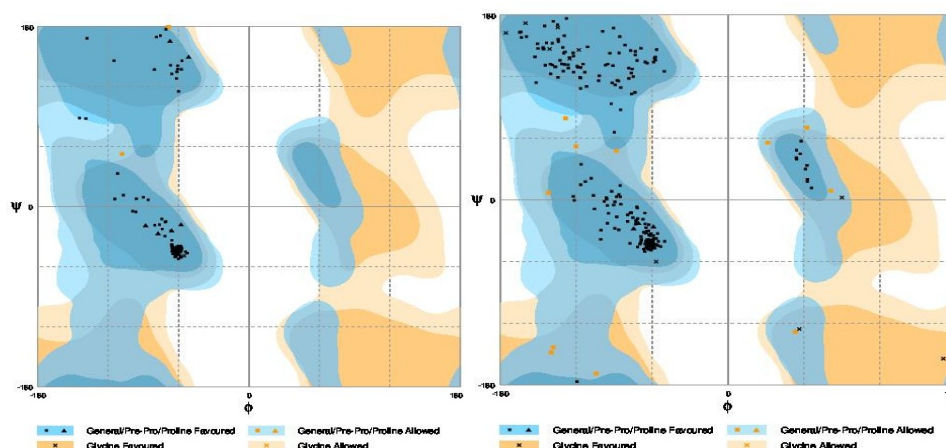
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Aminoglycoside O-nucleotidyltransferase ANT(4')-IaMBL fold metallo-hydrolase



Beta-lactam sensor/signaltransducerMecR1Immunoglobulin G-binding proteinA

In our study proteins Beta lactam sensor / signal transducer MecR1, Aminoglycoside O-nucleotidyl transferase ANT (4')-I a, MBL fold metallo-hydrolase, Oleate hydratase, and Immunoglobulin G-binding protein A are identified as membrane associated drug targets. The above mentioned proteins are not validated as drug targets they are putative by virtue of their essentiality using bioinformatics tools and deserve further studies.

## V. CONCLUSION

The large scale genome sequencing projects have increased the availability of completely sequenced genomic and proteomic data in public domain. Use of the DEG database is more efficient than conventional methods for identification of essential genes and facilitates the exploratory identification of the most relevant drug targets in the pathogen. The present study has thus led to the identification of several proteins that can be targeted for effective drug designing.

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