Identification and Characterization of Strain Specific Drug Target by Subtractive Genome Analysis of Methicillin Resistance *Staphylococcus aureus*

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In the present study, the idea was to utilize the strain specific available genome or proteome by using 'subtractive genomics approach' which helped in the identification and characterization of strain specific drug targets along with comparative analysis within two of the Methicillin resistance *Staphylococcus aureus* (MRSA) strains i.e., MRSA398 and MRSA252.The protocol involved various bioinformatics' tools and databases like similarity search between pathogen and host, essentiality study using the database of essential genes, metabolic functional association study using Kyoto Encyclopedia of Genes and Genomes Database (KEGG), cellular membrane localization analysis, drug bank database. Additionally, functional family characterizations of the identified non homologous hypothetical essential proteins were performed by using SVMProt server. Druggability potential of each of the identified drug targets were also evaluated by Drug Bank database. Our protocol resulted in identification and characterization of non-homologous/hypothetical and essential proteins which must not be homologous to the host genome. These non-homologous essential drug targets (6 and 21 for their respective stains) ensure the survival of the pathogen and hence can be targeted for drug discovery.

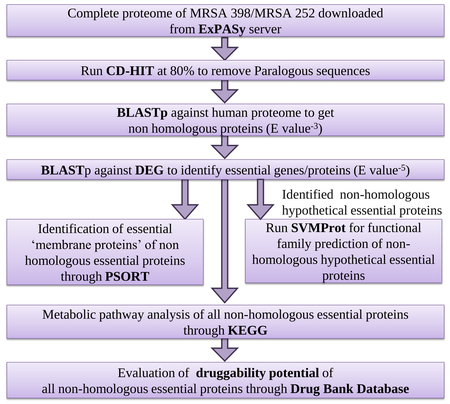
INTRODUCTION

• *Staphylococcus aureus* is one of the most out growing human bacterial pathogen in worldwide causing both nosocomial and community acquired infections.
• Pathogenicity: From mild skin infection to chronic fatal necrotizing pneumonia.
  ➢ In Children- Skin lesion, impetigo, bacteremia and abscesses.
  ➢ In Cattles- Bacteremia, Pneumonia meningitis, Endocarditis and mastitis.
• Conventional Treatment: It may include beta-lactam antibiotics, sulfa drugs, clindamycin and tetracycline.
• *S. aureus* acquire resistance to several antibiotics including powerful modern penicillin e.g. Methicillin, which define the subtype of bacteremia methicillin-resistance *S. aureus* (MRSA). Additionally, it was considered to increase the morbidity and mortality rate throughout the globe.
• MRSA typically has two strains i.e. MRSA 398 and MRSA 252 which have been reported as prudent for livestock-associated MRSA (LA-MRSA) for former and serious hospital acquired infections for later one.
• Prevalence: Health care associated *S. aureus* infections affiliated to MRSA has increased from 2% in 1974 to 64% in 2004.
• MRSA infections are asymptomatic in nature and can lead to life-threatening infection in bones and vital organ damage.
• Due to the emergence of antibiotic resistance strains, there is an essential need to develop strain specific drug targets to address the challenge of multidrug-resistant bacteria.

OBJECTIVES

To identify and characterize strain specific essential protein targets, which will eventually help in the identification of druggable proteins in two strains of MRSA by using different Bioinformatic tools and databases. We adopting a subtractive genome approach within the domain of Computational Biology.

METHODOLOGY



RESULTS AND DISCUSSION

❑ Our present study was to identify 'good drug targets' which are essential for pathogen survival but absent in human. In current strategy, we have identified non-homologous essential protein targets by using 'subtractive genomic approach' which may further lead to identify strain specific drug targets in comparative analysis of two strains of MRSA.
❑ **Table 1**: The overall outcome of our study.
❑ **Figure 1(a, b):** KEGG Metabolic pathway analysis of MRSA 398 and MRSA 252.
❑ **Figure 2**:Identification of essential membrane proteins through PSORT of both strains of MRSA.
❑ **Figure 3**:Functional family prediction of non homologous hypothetical essential proteins of MRSA 398 and MRSA 252 (SVMProt)
❑ **Table 2(a, b)**: The identified essential protein drug targets (Drug Bank Database) of MRSA 398 and MRSA 252.

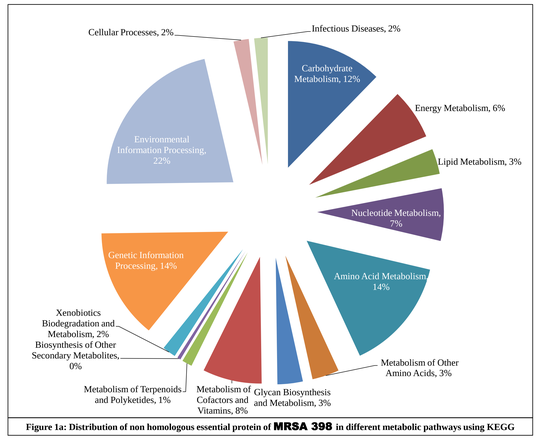


Figure 1a: Distribution of non homologous essential protein of **MRSA 398** in different metabolic pathways using KEGG

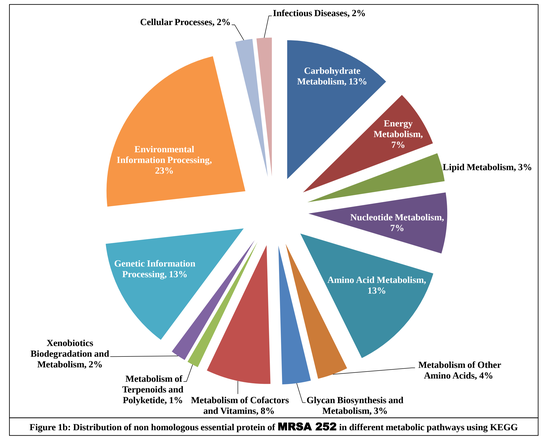


Figure 1b: Distribution of non homologous essential protein of **MRSA 252** in different metabolic pathways using KEGG

S.No.	PROTEINS	MRSA 398	MRSA 252
1	Total Number of Proteins	2648	2604
2	Paralogous removal by CD-HIT (>80% identical)	2598	2579
3	Number of proteins against *H. sapiens* using Blastp (E-value 10^{-3})	1826	1825
4	Essential proteins in DEG (E-value 10^{-5})	696	686
5	Essential proteins involved in metabolic pathways (KEGG)	464	452
6	Number of essential membrane proteins (PSORT)	214	219
7	Number of hypothetical protein as essential proteins (SVMProt)	46 out of 49	73 out of 75
8	Essential drug target proteins (DBD)	6	21

Table 1: Subtractive proteomic analysis results of MRSA 398 and MRSA 252

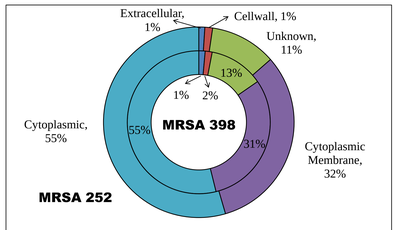


Figure 2: Essential membrane protein of MRSA 398 MRSA 252 using PSORT

CONCLUSION

We identified non-homologous essential proteins as potential drug targets against two strains of MRSA (i.e.398 and 252) by using subtractive genomic approaches that helped in the identification and characterization of non-homologous/hypothetical essential proteins in pathogen which has no homology with human and consequently can be used as strain specific novel druggable protein targets.

SNo.	Protein ID	Protein Name	DrugBank Target ID,Name	Drug Bank ID
1	2NPD	Probable nitronate monooxygenase	6877 Enoyl-(Acyl-carrier-protein) reductase	(DB08657)
2	DING	Probable ATP-dependent helicase dinG homolog	4276 DNA polymerase III Subunit epsilon	(Db01643)
3	NADD	Probable nicotinate-nucleotide	3087 Nicotinate-nucleotide adenylyltransferase	(DB04099)
			4469 Nicotinate-nucleotide adenylyltransferase	(DB01907; DB04272)
			3333 Nicotinamide mononucleotide adenylyltransferase	(DB01907; DB03227; DB04099)
			3296 Nicotinamide mononucleotide adenylyltransferase	(DB01907; DB02596; DB03227; DB04099)
4	Y1376	Probable tautomerase SAR1376 OS=Staphylococcus	2569 4-oxalocrotonate tautomerase	(DB02005)
5	Q6GJC7	Putative uncharacterized protein	3724 Protein methyltransferase hemK	(DB01752)
			4306 HemK protein	(DB01752; DB03473)
6	Q6GJ89	Putative uncharacterized protein	2652 Phosphomethylpyrimidine kinase	(DB02022)
			2670 Pyridoxamine kinase	(DB02153)
			3669 Ribokinase	(DB01936; DB03431; DB03909; DB04444)
7	Q6GGF5	Putative uncharacterized protein	5643 General secretion pathway protein E	(DB04395)
			4837 Cag-alfa	(DB02930; DB03431)
8	Q6GFY9	Putative uncharacterized protein	5440 UPF0067 protein yebR	(DB03814)
9	Q6GFR1	Putative uncharacterized protein	5497 N-acylamino acid racemase	(DB01646; DB02251;
			4278 N-acylamino acid racemase	DB03299; DB04511)(DB04167)
10	Q6GKQ3	Putative uncharacterized protein	3204 Thiosulfate sulfurtransferase glpE	(DB02761)
11	Q6GFW6	Putative uncharacterized protein	256 Tyrosyl-tRNA synthetase, cytoplasmic	(DB07817)
			6603 Phenylalanyl-tRNA synthetase beta chain	(DB00135;DB01766; DB03978; DB07205; DB08371; DB08617)
12	Q6GFU0	Putative uncharacterized protein	4309 S-adenosyl-L-methionine-dependent methyltransferase mraW	(DB01752)
13	Q6GDI4	Putative uncharacterized protein	4318 Siroheme synthase	(DB01752; DB01907; DB04522)
14	Q6GDK7	Putative uncharacterized protein	2573 Cocaine esterase	(DB01795; DB03793)
15	Q6GIE4	Putative uncharacterized protein	4404 FcbC1 protein	(DB01992; DB03613; DB04067;DB04242)
16	Q6GH54	Putative uncharacterized protein	4280 4-hydroxybenzoyl-CoA thioesterase	(DB01652;DB03613; DB04067)
17	Q6GF20	Putative uncharacterized protein	5314 UPF0079 ATP-binding protein HI0065	(DB03431)
18	Q6GJI4	Putative uncharacterized protein	3724 Protein methyltransferase hemK	(DB01752)
19	Q6GHL2	Putative uncharacterized protein	175 Thiamin pyrophosphokinase 1	(DB00152; DB04768)
20	Q6GHE5	Putative uncharacterized protein	4117 Probable pyruvate-flavodoxin oxidoreductase	
			4562 Pyruvate-ferredoxin oxidoreductase	(DB00507;DB01987; DB02410)
21	Q6GDT9	Putative uncharacterized protein	3107 Putative GTP pyrophosphokinase	(DB02836;

FUTURE DIRECTION

To identify the novel druggable targets which are essential for pathogenicity and survival of the MRSA. In addition to that we also look forward to identify effective drug candidates which act on those shortlisted druggable targets. As a result, block the essential metabolic pathways of the pathogen without altering the host mechanisms.

SNo.	Protein ID	Protein Name	DrugBank Target ID,Name	Drug Bank ID
1	D2N8Y1	Putative uncharacterized protein	5314 UPF0079 ATP-binding protein HI0065	(DB03431)
2	D2N980	Putative uncharacterized protein	3020 Sugar phosphatase supH	(DB04156)
3	D2NAA9	Putative uncharacterized protein	3020 Sugar phosphatase supH	(DB04156)
4	D2N7T5	Probable nicotinate-nucleotide	3087 Nicotinate-nucleotide adenylyltransferase	(DB01907; DB04272)
			4469 Nicotinate-nucleotide adenylyltransferase	(DB04099)
			3296 Nicotinamide mononucleotide adenylyltransferase	(DB01907; DB03227; DB04099)
			3333 Nicotinamide mononucleotide adenylyltransferase	(DB01907; DB03227; DB04099)
5	D2N379	Probable HMG-CoA synthase	4589 3-hydroxy-3-methylglutaryl CoA synthase	(DB02039; DB03059; DB03169)
			4601 HMG-CoA synthase	(DB02153; DB03059)
6	D2N4A3	Probable PTS system regulator	5694 Transcription antiterminator licT	(DB04530)

Table 2(a, b): The identified essential protein drug targets (Drug Bank Database) of MRSA 398 (a: on left) and MRSA 252 (b: on top)

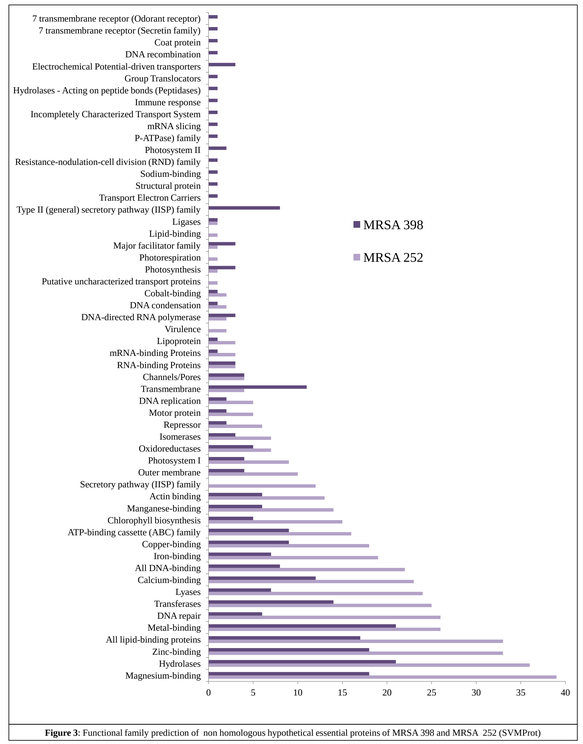


Figure 3: Functional family prediction of non homologous hypothetical essential proteins of MRSA 398 and MRSA 252 (SVMProt)

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