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Original Article

IDENTIFICATION OF PUTATIVE DRUG TARGETS IN MASTITIS CAUSING *STAPHYLOCOCCUS AUREUS* BY *IN SILICO* APPROACH

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ABSTRACT

Objective: In the present study an attempt has been made by the use of a computational approach to investigate putative drug targets in *Staphylococcus aureus*.

Methods: *In silico* comparative analysis of the metabolic pathways between the pathogen and the *Bos taurus* was carried out. Further detection of bacterial genes that are non homologous to host, but are essential for the survival of the pathogen represents a promising means of identifying novel drug targets. Metabolic pathways were obtained from the metabolic pathway database Kyoto Encyclopedia of Genes and Genomes (KEGG) and were compared to identify unique pathways present only in the pathogen and absent in the host.

Results: We have identified total 1930 proteins, which are non homologous to *Bos taurus* protein sequences and among them 374 enzymes are found to be essential for survival of the *S. aureus* according to the database of essential genes (DEG) database. Further, 10 proteins were predicted as cytoplasmic and cell wall associated proteins, which could serve as potential drug target candidates.

Conclusion: The identified potential drug targets form a platform for further investigation in discovery of novel therapeutic agents against *S. aur*eus.

Keywords: Mastitis, *Insilico*, *S. aureus*, DEG.

INTRODUCTION

Mastitis is an inflammatory disease of the mammary gland and is one of the most costly diseases of dairy animals which result in severe economic losses from reduced milk production, treatment cost, increased labor, withheld milk following treatment and premature culling of animals. The most common mastitis pathogens are found either in the udder as contagious pathogens or in the animal surroundings environmental pathogens such as in bedding and manure soil. Among the contagious pathogens, the most common is *S. aureus* [1]. It is considered as the main etiological agents responsible for mastitis and also responsible for significant economic losses all over the world [2]. In recent years, the incidence of antimicrobial-resistant in these bacteria has increased and is considered one of the reasons for low cure rates [3].

The process of drug design, development and commercialization is a tedious, cost-intensive and time-consuming process [4]. To overcome these challenges, several multidisciplinary approaches are required for the process of drug development. *In silico* drug designing is a form of computer-based modeling which is useful in drug discovery processes.

Identification of novel drug targets is required to develop new classes of drugs in order to overcome drug resistance and replace less efficacious treatments. The search for novel drug targets relies on the genomics data. The comparative and subtractive genomics approach can be used for selecting non homologous genes coding for proteins, which are present in pathogens but not in the host. This type of approach has been previously used in earlier studies for finding putative drug targets [5-7]. So the aim of the present study was to determine putative drug targets of *S. aureus* using *in silico* approach.

MATERIALS AND METHODS

To identify the essential genes of *Staphylococcus aureus*, the complete proteome sequence of *S. aur*eus was retrieved from NCBI. The proteins sequences were subjected to CD-HIT [8] analysis with a sequence identity cutoff of 0.8, to eliminate redundant sequences with more than 80% identity. The resultant proteins sequences were further used for the study. These sequences were further BLAST with the host genome i. e *Bos Taurus.* Proteins with an *E*-value (expectation value), 10^4 were eliminated, assuming that they have a certain level of homology with the host genome [9]. The resultant sequences had no homology with the host proteome. The next step was to identify the genes involved in the metabolic pathways in *S. aureus*. For this, the resultant non homologous protein to host was subjected to BLASTP analysis with a database of essential genes (DEG) which contain all the essential genes [10]. The sequencing thus obtained that could be considered as drug targets because they are not present in the host.

Further analysis was conducted on these essential proteins to determine their subcellular localization and function. The KAAS (KEGG Automatic Annotation Server) of the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to identify the functions [11]. The server provides functional annotation of genes by BLAST (Basic Local Alignment Search Tool) comparisons against the manually curated KEGG database. The program PSORTb V.3.0 [12] was used for subcellular localization prediction. Drug ability of the predicted 10 drug targets were further checked using Drug Bank. The drug Bank Database is a distinctive bioinformatics and chem informatics resource that combines detailed drug data with comprehensive drug target information.

RESULTS AND DISCUSSION

Development of novel drugs with potential therapeutic applications is one of the most complex and complicated process. It is quite obvious that increase of drug resistance properties requires more potential targets and by this *in silco* approaches increases the probability of success and also reduces the effort of the wet lab. In this present study we have tried to evaluate the targets could be a better target for rational drug designing.

The proteome of *S aureus* had a total of 2607 proteins. BLAST analysis of these proteins against the *Bos taurus* genome revealed

677 proteins that were homologous with the host genome and 1930 proteins are nonhomologous (table 1). Non homologous proteins as drug targets might lead to unwanted cross-reactions and cytotoxicity. However, this approach was also done to find potential drug targets in Bovine Herpes Virus 4 causing bovine mastitis [13]. Subtractive genomics approach is a powerful-method to identify the specific genes, which are present in the pathogen but absent in the host. Thus helps in the identification specific genes of an organism, which can be used as drug targets [14]. Comparative and subtractive genomics have been widely used for the prediction and identification of potential therapeutic targets and vaccine candidate proteins in numerous pathogenic bacteria and also fungi [5, 15, 16]. Hence, non homologous proteins were selected for further identification of essential genes using DEG database.

The functions encoded by essential genes are considered to constitute the foundation of life in the organism. It has been reported that essential gene products of microbial cells are promising new targets for antibacterial drugs [10]. Essential genes unique to an organism can be considered as species-specific drug targets [17]. DEG has been widely utilized by other researchers for identified of putative drug targets [18, 19]. 374 essential genes are found when BLAST against the DEG database (table 1).

This is in agreement with the experimentally determined essential genes 658 number [20] and through transposon-mediated differential hybridization identification of essential genes of 351 numbers in the *S. aureus* [21].

Table 1: Results of subtractive proteomic and metabolic pathway analysis for *S. aureus*

Total no. of proteins	2607
Nonparalogous (non homologous to host)	1930
Essential proteins in DEG	374
Essential proteins involved in metabolic pathways	278
Cell wall associated proteins	4
Cytoplasmic membrane associated proteins	6

Out of 374 essential proteins, 278 proteins were found to be involved in metabolic pathways. Computational prediction of bacterial protein localization provides a quick and inexpensive means for gaining insight in to protein function [22].

The protein localization study revealed that among the 278 proteins essentially involved in metabolic pathways, 6 proteins were present in cytoplasm and 4 is cell wall associated (table 2). Out of 10, two common potential drug targets were found to be highly similar to the target proteins in drug bank (table 3).

The computational genomics approach [23, 24] stated here, will speed up the drug discovery process by removing hindrances like dead-ends or toxicity that are encountered in classical approaches. We anticipate that future research based on this study will eventually provide interesting targets that can be successfully moved to drug development.

Table 2: Putative drug targets of *S. aureus*

Query ID	Protein function	Localization	DEG accession number	Gene reference	Length (AA)
Q2FDC2	Transfer complex protein TraG	Cell wall associated	DEG10170336	GI: 88196215	267
Q2FH04	Extracellular matrix-binding protein ebh	Cell wall associated	DEG10020122	GI: 15926817	1188
Q2FJH7	N-acetylmuramoyl-L-alanine amidase sle1	Cell wall associated	DEG10170336	GI: 88196215	267
Q2FIX4	CHAP domain family	Cell wall associated	DEG10170336	GI: 88196215	267
Q2FG20	Septation ring formation regulator EzrA	Cytoplasmic membrane associated	DEG10020122,DEG10170257	GI: 15926817,GI: 88195527	1188, 564
Q2FFM1	Monofunctional glycosyltransferase	Cytoplasmic membrane associated	DEG10170188	GI: 88195184	727
Q2FHQ5	Phospho-N-acetylmuramoyl-pentapeptide-transferase	Cytoplasmic membrane associated	DEG10170127,DEG10020107,DEG10170069	GI: 88194888,GI: 15926765,GI: 88194525	321, 321, 351
Q2FEQ9	Protein translocase subunit SecY	Cytoplasmic membrane associated	DEG10020258,DEG10170314	GI: 15927810,GI: 88196142	430
Q2FH76	Oxacillin resistance-related FmtC protein	Cytoplasmic membrane associated	DEG10170178	GI: 88195086	384
Q2FI21	Fmt protein	Cytoplasmic membrane associated	DEG10170108	GI: 88194754	397

Table 3: Drug ability of the predicted drug targets

Query ID	Drug bank target	Drug bank Accession no.	Putative drugs
Q2FFM1	Penicillin-binding protein 1A	DB01414, DB01327, DB00274, DB01328, DB01329, DB01331, DB00430, DB01333, DB00438, DB,01415, DB01332, DB00303, DB01598, DB04570	Cefacetrile, Cefazolin, Cefmetazole, Cefonicid, Cefoperazone, Cefoxitin, Cefpiramide, Cefradine, Ceftazidime, Ceftibuten, Ceftizoxime, Ertapenem, Imipenem, Latamoxef
Q2FI21	D-alanyl-D-alanine carboxypeptidase	DB03820, DB02514, DB04488, DB04340, DB00456, DB02136, DB03313, DB03450, DB01786, DB03843, DB01868, DB03927, DB02578	Cefalotin, Cephalosporin Analog,

In silico approach has been of great importance to develop fast and accurate target identification and prediction method for the discovery. Targets found are inevitable for the growth of the organisms. The current study can be carried forward to design a drug that can block these targeted proteins. Microorganisms are fast gaining resistance to the existing drugs. Thus designing better and effective drugs need a faster method.

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CONFLICT OF INTERESTS

Declared None

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