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Metabolic pathway analysis approach: Identification of novel therapeutic target against methicillin resistant *Staphylococcus aureus*

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ABSTRACT

Multiple Drug Resistant (MDR) bacteria are no more inhibited by the front line antibiotics due to extreme resistance. Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the MDR pathogens notorious for its widespread infection around the world. The high resistance acquired by MRSA needs a serious concern and efforts should be carried out for the discovery of better therapeutics. With this aim, we designed a comparison of the metabolic pathways of the pathogen, MRSA strain 252 (MRSA252) with the human host (i.e., *Homo sapiens*) by using well-established in silico methods. We identified several metabolic pathways unique to MRSA (i.e., absent in the human host). Furthermore, a subtractive genomics analysis approach was applied for retrieval of proteins only from the unique metabolic pathways. Subsequently, proteins of unique MRSA pathways were compared with the host proteins. As a result, we have shortlisted few unique and essential proteins that could act as drug targets against MRSA. We further assessed the druggability potential of the shortlisted targets by comparing them with the DrugBank Database (DBD). The identified drug targets could be useful for an effective drug discovery phase. We also searched the sequences of unique as well as essential enzymes from MRSA in Protein Data Bank (PDB). We shortlisted at least 12 enzymes for which there was no corresponding deposition in PDB, reflecting that their crystal structures are yet to be solved! We selected *Glutamate synthase* out of those 12 enzymes owing to its participation in significant metabolic pathways of the pathogen e.g., Alanine, Aspartate, Glutamate and Nitrogen metabolism and its evident suitability as drug target among other MDR bacteria e.g., *Mycobacteria*. Due to the unavailability of any crystal structure of *Glutamate synthase* in PDB, we generated the 3D structure by homology modeling. The modeled structure was validated by multiple analysis tools. The active site of *Glutamate synthase* was identified by not only superimposing the template structure (PDB ID: 1E0A) over each other but also by the Parallel-ProBiS algorithm. The identified active site was further validated by cross-docking the co-crystallized ligand (2-oxoglutaric acid; AKG) of PDB ID: 1LLW. It was concluded that the comparative metabolic in silico analysis together with structure-based methods provides an effective approach for the identification of novel antibiotic targets against MRSA.

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1. Introduction

Since existing antibiotics are ineffective against Methicillin Resistant *Staphylococcus aureus* strain 252 (MRSA252), the discovery of novel antibiotics is of prime need. Unfortunately, the fast growing resistance among MRSA is a major obstacle for bringing new regimens. Contrary to that the recent developments in complete genome analysis with the combination of bioinformatics exemplify a simple method for searching the unique therapeutic targets (Butt et al., 2012a). In silico comparative metabolic pathway analysis is a well-established method having the following applications:

1 Cross species metabolic pathway comparison (Ebenhoh et al., 2005).
2 Identification of unique metabolic pathways and related enzyme data (Amir et al., 2014).
3 Identification of common pathways between host and pathogen (Smith et al., 2012).

The availability of full metabolic pathways and its related enzymes opens up higher order possibilities for comparison analysis (Bork

Abbreviations: MRSA, Methicillin Resistant *S. aureus*; KEGG, Kyoto Encyclopedia of Genes and Genomes; PDB, Protein Databank; BLAST, Basic Local Alignment Search Tool; NCBI-GI, NCBI Gene Identification Number; MDR, Multiple Drug Resistant; TB, Tuberculosis; MTB, *Mycobacterium tuberculosis*.

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et al., 1998). The identification of unique metabolic pathways and corresponding essential proteins is interesting for targeting biochemical reactions and as well as of pharmacological discovery of successful therapeutic candidates in the future. Though there are similar literature reports available with *S. aureus* (Haag et al., 2011; Uddin and Saeed, 2014), however, none of the literature described the complete metabolic pathway comparisons and subsequent drug target selection. Whereas other approaches emphasized more on the genomics information for the comparison, our methodology is more focused on the metabolic pathways of the pathogen. An in silico comparative metabolic pathway analysis approach was adapted in the current study and novel therapeutic targets against MRSA were proposed. Those identified novel therapeutic targets could then be passed on to a drug discovery campaign for the discovery of novel therapeutic regimes. The aim of the current study is to propose those targets which are essential for the survival of the pathogen and at the same time should not have homology with the human host. It was expected that our results would facilitate the selection of MRSA drug targets which could enter into a successful drug design pipeline.

2. Materials and methods

The methodology comprised of the following well defined steps:

2.1. Comparative metabolic pathway analysis

Firstly, the complete metabolic pathway information and assigned IDs of MRSA252 (sar) and the host *Homo sapiens* (hsa) were retrieved from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database [version 66.1, May 1, 2013 (Kanehisa et al., 2006)]. The KEGG is considered as one of the comprehensive sources of metabolic pathway information, and is periodically updated. Comparing the metabolic pathways of MRSA252 with the host helped to identify only the unique pathways of the pathogen, i.e., absent in the human host whereas the remaining pathways were considered as common. Enzymes involved in the unique pathways and their respective protein sequences were then identified from KEGG database and subsequently retrieved from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/).

2.2. Subtractive genomics and prioritization of therapeutic targets

A subtractive genomics approach was further applied only on the unique protein sequences using the Database of Essential Genes [DEG; version 7.0 (Zhang et al., 2004)] comprising of 13,783 essential genes of more than 20 bacterial pathogens including *S. aureus* with BLASTp (Camacho et al., 2009) at an *e*-value of 10^{-5}. The DEG would make sure that we precede only with the essential protein :sequences which cut off the side effects when targeted by drug candidates. Identified essential protein sequences were subjected to BLASTp against non-host proteome database at an *e*-value of 5×10^{-3} to get unique non-host (non-homologous) essential protein sequences. Additionally, all non-host essential protein sequences were subjected to DrugBank Database [DBD; version 3.0 (Knox et al., 2011)] using BLASTp at an *e*-value of 10^{-3} which resulted in the identification of potential drug targets. We further applied PSORTb tool [version 3.0.2 (Nancy et al., 2010)] on prioritized protein drug targets in order to predict the subcellular localization of the shortlisted sequences. PSORTb provides five localization sites and the associated probability value for each module using Bayesian network. The criterion is based on *p*-value > 7.5 that is considered to be a good cutoff. Higher value reflects more confidence that the given protein is present in that subcellular location. If none of the localization sites have a score >7.5, a prediction of 'unknown' is regarded. The subcellular localization is an important indicator of the possibility of potential vaccine candidates (Mitchell et al., 2004).

2.3. Homology modeling, binding site detection and its validation by docking

In the end, we looked for the existence of any Protein Data Bank [PDB; (Berman et al., 2014)] entry for the potential drug proteins of MRSA252 using BLASTp similarity search at an *e*-value of 10^{-3}. Only twelve catalytic proteins (enzymes) were shortlisted as no protein structure is available in PDB. *Glutamate synthase* was selected for structure-based method approaches (see Results for more details). We retrieved the sequence of the large subunit of *Glutamate synthase* from UniProt (UniProt, 2014) with NCBI reference ID: YP_039920 (UniProt ID: Q6GJK2). For template selection, BLASTp search against PDB at an *e*-value of 10^{-3} retrieved PDB ID: 1EA0 (the large α subunit of *Glutamate synthase* from *Azospirillum brasilense* with 41% identity and 59% similarity) (Binda et al., 2000). Fig. S2 shows the sequence alignment between the query sequence and the template. The 3D model of *Glutamate synthase* was generated using MODELLER [version 9.12 (Webb and Sali, 2014)]. Stereochemical quality of generated model was verified by PROCHECK (Laskowski et al., 1993) and ProSA (Wiederstein and Sippl, 2007). Both are well-established single (or multiple) model protein structure analysis tools. The *z*-score calculated by ProSA exhibits the model quality while measuring the deviation of the total energy if the structure with respect to an energy distribution derived from random conformations. The negative *z*-score of homology model is mandatory to acquire the good quality model. The Root Mean Square Deviation (RMSD) between the modeled protein and template was calculated by MatchMaker tool in CHIMERA [version 1.6 (Pettersen et al., 2004)]. We further identified the binding site of the modeled enzyme by the ProBis binding site tool [version 2012 (Konc and Janežič, 2012)]. In ProBis, the structurally similar binding sites are determined by comparing the query sequences with the non-redundant protein database. The ProBis then ranked highest binding site similarity sequences as top.

Subsequently, docking was performed by GOLD docking program (Verdonk et al., 2003) based on genetic algorithm. For docking, we extracted the native ligand of PDB ID: 1LLW (van den Heuvel et al., 2002) as suggested by ProBis. The atom-type corrections of the ligand were performed by SYBYL (version 7.3, http://www/tripos.com), and the ligand structure was energetically minimized by the conjugate gradient method until the convergence is achieved. RMSD between the docked conformation and the co-crystallized ligand was calculated by in-house script. Finally, the 2Dligand interaction diagram of the ligand docked within the binding site of modeled *Glutamate synthase* was drawn by Molecular Operating Environment (MOE; http://www.chemcomp.com/).

3. Results

The current study is an application of advanced comparative metabolic pathway analysis. The study was further augmented to subtractive genomics leading to structure-based function and structural characterization of the potential pharmacological target. A workflow of the current study was defined in Fig. 1. The unique and essential metabolic pathways are significant for bacterial growth, e.g., regulation of bacterial nutrient uptake, pathogenicity and other biochemical processing. A search for the inhibitors of the proteins belonging to the unique and essential pathways is therefore considered as a promising approach to deal with the challenging MRSA infection.

3.1. Comparative metabolic pathway analysis

The basic aim of this step was to perform a comparison between the complete metabolic pathways of MRSA252 and its human host which eventually resulted in listing the unique as well as the common metabolic pathways. Subsequent retrieval of the protein sequences of only the unique metabolic pathways would lead to prioritized potential drug targets.

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