Research Article ISSN 2689-1050

Chemical & Pharmaceutical Research

Two-Component System Transcriptional Regulator PrrA & Transcriptional Regulator KdpE Revealed as Novel Drug Targets in the Pan-Proteome Analysis of *Mycobacterium tuberculosis* CCDC5180

Kunal Gharat[1,2], Vikas Jha[1*], Divya Nikumb[1], Joshua Koli[1], Vrushali Dhamapurkar[1], Omkar Parulekar[3], Diksha Poojari[1], Simeen Rumani[1], Aparna Sahu[1], Shivani Kore[1] and Farzha Khan[4]

[1]*National Facility for Biopharmaceuticals, Guru Nanak Khalsa College of Arts, Science & Commerce, Mumbai-19, Maharashtra, India.*

[2]*Department of Biochemistry and Molecular biology, Faculty of Biology and Chemistry, University of Bremen, Bibliothekstraße 1, 28359 Bremen, Germany.*

[3]*Department of Microbiology, St. Xavier's College, 5, Mahapalika Marg, Dhobi Talao, Chhatrapati Shivaji Terminus Area, Fort, Mumbai, Maharashtra-400001, India.*

[4]*Department of Biological Sciences, SVKM's NMIMS Sunadan Divatia School of Science, Vile Parle West-56, Mumbai, Maharashtra, India.*

***Correspondence:**
Mr Vikas Jha, National Facility for Biopharmaceuticals, Guru Nanak Khalsa college, Mumbai 400019 India, E-mail: vikasjjha7@gmail.com.

Received: 17 Sep 2022; **Accepted:** 25 Oct 2022; **Published:** 30 Oct 2022

Citation: Gharat K, Jha V, Nikumb D, et al. Two-Component System Transcriptional Regulator PrrA & Transcriptional Regulator KdpE Revealed as Novel Drug Targets in the Pan-Proteome Analysis of *Mycobacterium tuberculosis* CCDC5180. Chem Pharm Res. 2022; 4(4): 1-11.

ABSTRACT

Multidrug-resistant bacteria are now widely recognized as a global threat and a substantial public health concern. Antimicrobial resistance (AMR) is known as a more significant threat to humanity. Multidrug-resistant (MDR)/ Extensively Drug-resistant (XDR) Mycobacterium tuberculosis (M. tb) is a prime example of AMR. Furthermore, treating MDR-TB is more challenging since it demands second-line treatments, which have severe side effects. Genomic & proteomic approaches are employed in pharmaceutical research to identify novel drug targets. A subtractive proteomics approach was applied in this study to identify promising therapeutic targets for the MDR strain M. tb CCDC5180. The subtractive proteome approach of the whole proteome of M. tb CCDC5180 showed a list of 14 essential, cytoplasmic, and unique metabolic proteins discovered to be druggable. Among these 14, only eight proteins were involved in the pathogen's virulence. Finally, two proteins, PrrA and KdpE, were identified as potential novel drug targets, and further docking of these proteins with phytochemicals resulted in two promising compounds Cepharanthine and Alianthone against these novel targets.

Keywords

Mycobacterium tuberculosis CCDC5180 (*M. tb* CCDC5180), Multidrug-resistant (MDR), Extensively Drug-resistant (XDR), Novel drug target, Subtractive proteomics approach.

Abbreviations

M. tb: *Mycobacterium tuberculosis*, TB: Tuberculosis, AMR: Antimicrobial resistance, MDR: Multidrug-resistant, XDR: Extensively Drug-resistant, DEG: Database of essential genes, NCBI: National Center for Biotechnology Information, CD-HIT: Cluster Database at High Identity with Tolerance, KEGG: Kyoto encyclopaedia of Genes and Genomes, VFDB: Virulence factor database, VFs: Virulence factors, PDB: Protein Data Bank, Tat: Twin-arginine translocation, LAM: Lipoarabinomannan, TCSs: Two-component systems, QS: Quorum sensing, HK: Histidine kinase, RR: Response regulator, PPI: Protein-protein interactions,

Erp: Exported repetitive protein, Fbp: Fibronectin-binding protein, CID: Compound Identifier.

Introduction

One-third of the world's population is infected with *Mycobacterium tuberculosis* (*M. tb*), the primary cause of tuberculosis (TB). TB is one of society's oldest and deadliest diseases, placing a burden on health, society, and the economy [1]. More than hundreds of years ago, Robert Koch identified *M. tb* as the causative agent of human tuberculosis [2]. Even in areas where the disease was supposed to be eradicated, TB is still a serious threat to civilization and has returned in the past 30 years [3]. Most of the time, TB affects the lungs, but it can also harm the brain, intestines, kidneys, or spine. The location of the TB bacteria in the body affects the TB symptoms. Pulmonary TB can manifest as symptoms including a persistent cough, chest pain, hemoptysis, weakness or weariness, weight loss, fever, and night sweats [4]. Despite numerous "eradication" efforts over the years, tuberculosis still affects about one-third of the population, with between 5% and 15% of people experiencing an active illness at some point in their life. According to several decades of histology data from *in vivo* infections in people and animal models, the macrophage is the main host cell for most of the infection cycle before the release of bacteria into the cavitating lesion. Understanding the basis of *M. tb* survival within the phagocyte is thus one of the key issues in determining how *M. tb* has proven to be so successful in causing infection [5]. In recent years, major advances have been made in understanding the molecular causes of mycobacterial pathogenicity, virulence, and persistence. The discovery of critical mycobacterial virulence genes has made a significant contribution. The substantial investigation of virulence genes made possible by the use of transposon mutant libraries in conjunction with diverse *in vivo* screening techniques has led to the clarification of the mechanisms by which bacilli survive and persist in their hosts [6]. Most of these virulence genes encode for cell surface proteins, signal transduction system regulators, and enzymes involved in the lipid pathway. Mycobacteria lack traditional virulence characteristics like toxins, which are present in other bacterial infections, but tuberculosis pathogenicity is strongly influenced by the patient's immune response [7].

Drug-resistant *M. tb* strains are a severe healthcare concern as they necessitate the use of certain second-line drugs that may be less effective, more expensive, and toxic, as well as an advanced infrastructure for drug susceptibility testing that is not easily accessible in resource-constrained settings. Only 54% of MDR and 28% of extremely drug-resistant (XDR—MDR plus resistance to fluoroquinolones and any second-line injectable aminoglycoside/cyclic peptide) patients are cured, compared to 83% of drug-susceptible patients [8]. The CCDC5180 strain discovered in 2004 was found to be resistant to four first-line drugs. CCDC5180, the multidrug-resistant isolate, has a genome size of 4,405,981 bp and is highly conserved. Genomic annotation data revealed three types of virulence factors, one of which is involved in lipid metabolism and signaling. The ability of the pathogen to escape or infiltrate host macrophages is facilitated by the cell envelope first, and then cell wall-associated genes (including dim and pks). First-line drug resistance genes such as katG, inhA, ahpC, kasA, and ndh for INH resistance, rpoB for rifapentine resistance, embB for ethambutol (EMB) resistance, and rpsL and rrs for streptomycin resistance were discovered in its genome [9]. There lies an urgent need for new treatment regimens, drugs, and diagnostics to slow the evolution of drug resistance, limit the transmission of resistant variants, and improve the treatment outcome of patients infected with MDR/XDR *M. tb* strains.

With the emergence of drug resistance, modern techniques for developing novel therapeutic agents find use. The subtractive genomic technique, in particular, is one key technique, that has been widely utilized to anticipate novel therapeutic targets against infectious diseases [10]. Subtractive genomics is modern in silico approach which is employed to identify new drug targets based on the determination of essential and non-homologous proteins within the pathogenic organism [11]. In the subtractive genomics approach, the host and pathogen genomes are compared, and the non-host pathogen's unique and essential proteins are proposed as drug targets that are vital to the pathogen's survival [12]. This approach involves the subtraction of proteins or genes between the host and pathogen and provides information on a set of proteins that are likely to be essential to the pathogen but are absent in the host [13]. Potential therapeutic drug targets for various harmful bacteria have been developed using the subtractive proteomics technique; thus, this approach is beneficial in developing drug candidate for *M. tb* [14] .

Hosen et al. used a similar approach to uncover potential drug targets against the *M. tb* F11 strain. The group's findings resulted in fifteen proteins that might be exploited as therapeutic targets to treat the disease [15]. Uddin et al. employed a comparative subtractive genomics methodology to identify drug targets in three *Mycobacterium avium* subsp. *Hominissuis* strains, to narrow down potential drug targets; the entire proteome was systematically subtracted. The entire dataset of proteins from *Mycobacterium avium* subsp. *hominissuis* was obtained for this purpose. The subtractive genomics method was used, which involved a homology search between the host and the pathogen to subtract non-druggable proteins, resulting in the identification of a few prioritised potential drug targets against the three strains of *M. avium* subsp. *Hominissuis*, namely, MAH-TH135, OCU466, and A5. The authors in this study employ the use of subtractive proteomics to identify novel drug targets in against MDR strain *M. tb* CCDC5180.

Materials and Methods

Retrieval of Proteomes of Host and Pathogen

The whole proteome of *M. tb* CCDC5180 [9] was downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore/CP002885.1), while the complete proteome of *Homo sapiens* (Accession Id: UP000005640) was retrieved from the Uniprot database (https://www.uniprot.org/). The essential protein sequences of *M. tb* H37Rv, *M. tb* H37Rv II and were retrieved from the database of essential genes (DEG) (http://www.essentialgene.org/) [16].

Removal of Paralogous/ Duplicate sequences

Large biological sequence datasets can be clustered and compared using the commonly known tool CD-HIT. Subtractive analysis of the entire proteome of *M. tb* CCDC5180 was carried out using Cluster Database at High Identity with Tolerance (CD-HIT) (http://weizhong-cluster.ucsd.edu/cd-hit/) [17]. CD-HIT tool helps in the identification of paralogs and duplicate sequences. The entire proteome of *M. tb* CCDC5180 was purged at a 60% threshold. The CD-HIT tool categorizes protein or nucleotide sequences into clusters, reducing redundancy and manual intervention in sequence analysis. The remaining proteins were classified as orthologous sequences.

Identification of Non-Homologous Proteins

To avoid adverse responses in humans during drug therapy, the drug targets must be non-homologous to human proteins. The NCBI BLASTp tool (E-value 1×10^{-4}) was used to compare the *M. tb* proteome to the human proteome in order to identify non-host homologous targets [18]. The standalone BLAST version 2.8.1 was downloaded from the NCBI FTP server (https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/). For the identification of the non-human homologous proteins, the non-paralogous sequences were subjected to BLASTp against the *H. sapiens* proteome with a threshold expectation value (E-value) of 10^{-4} and the sequence identity was >25%. The protein sequences showing no homology with the human host were selected for further analysis.

Identification of Essential proteins

Using a genome-wide gene essentiality study, the database of essential genes (DEG) (http://origin.tubic.org/deg/public/index.php) identifies essential protein-coding genes. The database of essential genes is made up of 646 non-coding RNAs, promoters, regulatory regions, replication origins, and 22,343 essential protein-coding genes and proteins from 31 prokaryotes and 10 eukaryotes that are experimentally identified [19]. It is a database that provides listings of genes and their associated sequences required for bacterial viability. To filter out proteins that appeared to be essential, a standalone BLASTp analysis was performed against non-homologous protein sequences with an E-value of 10^{-4} and a minimum bit score cut-off of 100.

Localization of the Essential Proteins

One of the best tools for predicting bacterial localization is PSORTb (https://www.psort.org/psortb/). Multiple analytical modules are included in PSORTb 3.0, and each one is performed separately [20]. By examining one biological parameter known to affect the subcellular location, each module confirms the specific localization site. The ultimate prediction is created by integrating all the results. A final score indicates the likelihood of a protein belonging to a particular site and after examining the multiple site, PSORTb will produce the most likely localization site [21]. A protein can be found in one of five places: 1) the cytoplasm, 2) the inner membrane, 3) the periplasm, 4) the outer membrane, and 5) the extracellular space. Outer membrane or extracellular proteins can be used as vaccine candidates, whereas cytoplasmic proteins can be used as potential therapeutic targets. Using this tool, we can obtain the localization information of the essential proteins of *M. tb*.

Metabolic Pathway Analysis

KEGG (Kyoto encyclopaedia of gene and genome) is a pathway database [22]. The KEGG database was used to perform metabolic pathway analysis of the essential proteins screened to identify unique potential therapeutic targets. The BioCyc database [23] was used to find unique metabolic pathways found exclusively in the pathogen. The pathogen's remaining pathways were classified as common since they were found in the host's metabolism.

Druggability and Virulence Factor Analysis of Cytoplasmic Proteins

The druggability of cytoplasmic proteins participating in specific metabolic pathways was determined against all drug targets present in the Drug Bank database [23] . Drug targets with a bit score of more than 100 and an E value of less than 0.005 were identified as potential drug targets. One key aspect in determining therapeutic targets is virulence factors (VFs). Bacterial pathogens release VFs, which help them modify or impair host defensive mechanisms, resulting in illness. Virulence factor database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi) is a database that contains offensive, defensive, non-specific, and virulence-associated regulated proteins from 25 pathogenic bacteria [24]. The shortlisted druggable proteins were subjected to BLASTp search against the protein sequences from the VFDB core dataset (A and B) with default hit with cut-off bit score >100, and E-value was 10^{-5}.

Protein-Protein Interaction Network Analysis

STRING is a database of known and predicted protein interactions containing both direct (physical) and indirect (functional) relationships obtained from various sources [25]. Protein-protein interaction of the virulent proteins was carried to find out the potential metabolic functional associations among all identified proteins through the protein interaction database STRING.

Docking Analysis

The three-dimensional structure of the protein PrrA and KdpE considered a novel therapeutic target against *M. tb* CCDC5180 was obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/). The details of these proteins are mentioned in Table 2. The Phytochemicals showing antitubercular properties were retrieved from Dr. Dukes Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov/phytochem/search). The PubChem library (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the three-dimensional structure of these phytochemicals. Proteins were pre-processed in Discovery Studio 2020 prior to molecular docking analysis [26]. This step involves the removal of any hetero-groups as well as the addition of Hydrogen atoms and various charges. In addition, the PyRx tool was used to prepare ligands and receptors in the PDBQT file format. PyRx was used for molecular docking to better understand the interaction between receptors and ligands. Blind docking was performed by

covering the whole protein molecule. The molecule showing the lowest binding energy was selected and screened according to the Lipinski rule of five, the molecule showing deviation from the given set values was eliminated by using this tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

Results and Discussion

Removal of Hypothetical Proteins and Paralogous Sequences after Proteome Retrieval

The whole proteome of *M. tb* CCDC5180 was retrieved in FASTA format from the NCBI database. The total proteome count was found to be 4160, with 1602 of them being hypothetical proteins. Hypothetical proteins are proteins whose functions are unknown and can be challenging to comprehend without any experimental evidence [27] Hence, these 1602 hypothetical proteins were excluded from the current analysis. Furthermore, the CD-HIT tool was used to remove paralogous sequences from 2558 annotated proteins. Out of 2558 proteins, 187 sequences were identified as paralogous using the CD-HIT algorithm with 60% identity. A 40 percent sequence identity cut-off has been broadly accepted [28] Duplicate sequences should be eliminated since, in most circumstances, utilizing similar sequences causes unwanted biases. The CD-HIT grouped the paralogous sequences, reducing the overall number of sequences in the process.

Identification of Non-Homologous Proteins

Protein sequences exclusively found in pathogens and not in the host were separated by using a subtractive methodology. A total of 2371 non-paralogous proteins identified by the CD-HIT tool were subjected for BLASTp against the complete human proteome with a threshold E-value 10^{-4}. This step is crucial for increasing the drug's specificity against the pathogen while also preventing cross-reactivity with a host protein [29]. The only sequences that were retained were those that showed sequence similarity of less than 30% or no similarity with the human proteome, and a total of 1389 non-homologous proteins were identified in *M. tb* CCDC5180.

Identification of Essential Proteins

Essential gene products are critical for the survival of pathogens and are frequently required to sustain their basic cellular processes [30] Thus, the inactivation of these essential proteins could be lethal to the microorganism, making it a viable target for therapeutic measures. The Database of Essential Genes contains information on essential genes discovered by experimental approaches in Gram-positive and Gram-negative bacteria. A standalone BLASTp search for the non-homologous proteins was done to screen out the essential proteins with a threshold E-value 10^{-4} and a minimum bit score cut-off of 100. A total of 425 proteins satisfied the criteria and were considered to be essential.

Localization of the Essential Proteins

A critical part of identifying efficient and acceptable therapeutic targets is the localization of proteins inside the cell [31]. In addition, protein localization aids genomic annotation, cytobiology, and proteomics [32]. Cytoplasmic proteins can be used as drug targets, while extracellular proteins can be used as vaccine candidates. The prediction of subcellular localization of the essential proteins was achieved by PSORTb 3.0. The findings revealed that 230 (54%) of the 425 essential proteins were found in the cytoplasm, while 141 (33%) were found in the cytoplasmic membrane. The remaining proteins were found in various locations, with 4 (1%) and 3 (1%) proteins found in the extracellular and cell wall regions. Moreover, 47 (11%) proteins were identified as unknown due to the tool's ability to predict proteins in multiple sites at the same time. The distribution of proteins by PSORTb is graphically shown in Figure 1.

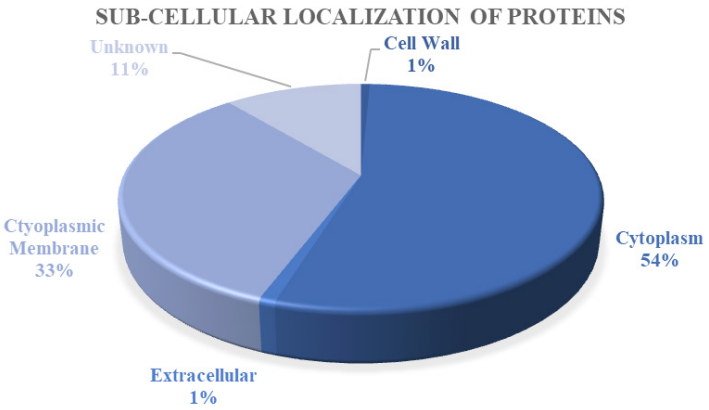

Figure 1: Graphical representation of the localization of proteins in different compartments of the cell.

Metabolic Pathway Analysis

The KEGG database was used to analyse the metabolic pathways of the 425 essential proteins. 218 of the 425 protein sequences were found to be engaged in the organism's metabolic activities. The remaining 207 proteins were not engaged in any metabolic activities; however, a few proteins were identified as drug transporters, iron channel regulators etc. As a human pathogen, bacteria undertake all of the same basic metabolic reactions as a human cell does. However, they may exhibit a variety of metabolic reactions that are not found in human or eukaryotic cells. Such metabolic reactions that are found exclusively in pathogens are known as Unique metabolic pathways (UMP). Moreover, the proteins involved in this UMP can serve as a favourable drug target [12]. Using the BioCyc database, each pathway was scrutinized with the goal of identifying UMP. The findings revealed that 59 proteins were engaged in the following UMP: Biosynthesis of siderophore group non-ribosomal peptides, Bacterial secretion system, D-Alanine metabolism, Lysine biosynthesis, Histidine metabolism, Phenylalanine, tyrosine, and tryptophan biosynthesis, Peptidoglycan biosynthesis Arabinogalactan biosynthesis – Mycobacterium, Lipoarabinomannan biosynthesis, Polyketide sugar unit biosynthesis, Two-component system and Quorum sensing (Figure 2).

Non-ribosomal peptides are a wide group of natural compounds that belong to the category of secondary metabolites and have a variety of features such as toxins, siderophores, pigments, and antibiotics. Since iron functions as a cofactor for various critical metabolic pathways, it is one of the essential nutrients for almost all bacteria. It has been proven that bacteria cannot survive in

an iron deficit medium. Therefore, bacteria use a siderophores-mediated mechanism to obtain iron from the host to overcome the iron deficit. Mycobactin biosynthetic pathway is one such type of siderophore pathway, and therefore, the proteins involved in this pathway can be used as a potential drug target [33] . Another ideal drug target would be the proteins associated with the bacterial secretion system. Bacteria move proteins across the cytoplasmic membrane using the bacterial secretion system, specifically the twin-arginine translocation (Tat) pathway. Moreover, a functional Tat pathway is vital for virulence, survival, and other physiological processes during infection [34] . Bacterial amino acid metabolism is diverse, and it plays a pivotal role in supporting bacterial growth. D-alanine, which is synthesized via D-alanine metabolism, is required as a precursor in the bacterial peptidoglycan biosynthesis pathway. Interestingly, the enzymes involved in this metabolism are primarily found in prokaryotes and not in eukaryotes suggesting that they could be used as a therapeutic target [35] Alanine racemase is involved in D-alanine metabolism, while Putative tetrahydro dipicolinate N-succinyltransferase, Succinyl-diaminopimelate desuccinylase, and Diaminopimelate epimerase are involved in lysine biosynthesis. Furthermore, histidine and phenylalanine, tyrosine, and tryptophan biosynthesis have been reported as an ideal drug target against *M. tb* [36].

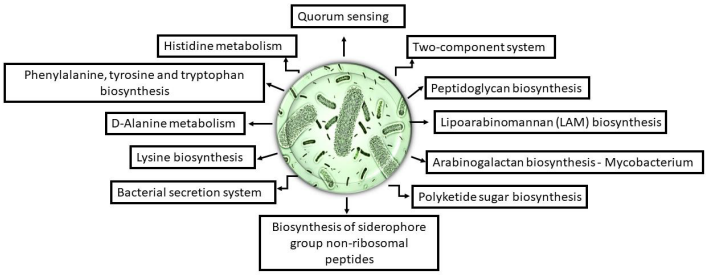

Figure 2: List of unique metabolic proteins involved in *M. tb* CCDC5180

The peptidoglycan layer is critical for cell wall structural integrity, especially in Gram-positive bacteria, and it also contributes to pathogenicity by providing resistance to osmotic lysis [37]. The rupture of the pathogen's cell wall might result in death; hence, enzymes involved in peptidoglycan production could be an effective drug target. Arabinogalactan biosynthesis – Mycobacterium pathway is responsible for the mycobacterial cell wall formation, and the presence of mycolyl–arabinogalactan–peptidoglycan (mAGP) complex aids *M. tb* in evading the host immune system and even conventional antibiotics [38]. Two of the four first-line anti-Tb regimens, isoniazid and ethambutol, target mycolic acid and arabinogalactan synthesis, respectively [39]. Arabinosyl transferase A, Arabinosyl transferase B, Putative dTDP-RHA: A-D-GlcNAc-diphosphoryl polyprenol, A-3-L-rhamnosyltransferase WbbL1, Glycosyl transferase, Putative transmembrane protein, and Bifunctional udp-galactofuranosyl transferase are present in *M. tb* CCDC5180 Arabinogalactan biosynthesis.

Lipoarabinomannan (LAM) synthesized via the LAM biosynthesis pathway is associated with *M. tb* pathogenicity [40]. It plays a pivotal role in *M. tb* infection by inhibiting T cell proliferation and deactivation of IFN-γ [41] Targeting it might be a potential strategy because of its importance in *M. tb* infection. The proteins taking part in the *M. tb* CCDC5180 LAM biosynthesis are Putative integral membrane protein, Arabinosyl transferase C, Putative transmembrane protein, Mannosyltransferase, Alpha-mannosyltransferase PimA, and Lipid A biosynthesis lauroyl acyltransferase.

The polyketide sugar unit biosynthesis pathway synthesizes a plethora of compounds with a broad spectrum of structure and function. L-rhamnose is a crucial saccharide found in both Gram-positive and Gram-negative bacteria, and it plays a significant role in the virulence of a variety of pathogenic bacteria, including *M. tb* [42] In addition, the dTDP-L-rhamnose pathway is also involved in mycobacterial cell wall synthesis. As a result, targeting enzymes in this pathway might disrupt the mycobacterial cell's integrity, which is vital for growth and survival inside the host [43] .

Two-component systems (TCSs) are ubiquitous in all three domains of life: Eukarya, Bacteria, and Archaea. TCSs are regulatory mechanisms in bacteria that frequently influence the adaptive response to various stimuli. A sensor protein called histidine kinase (HK) and a response regulator (RR) makes up the majority of them [44]. Motility and chemotaxis, sporulation, biofilm formation, and quorum sensing are just a few of the cellular functions that TCSs may control [45]. TCSs have an important role in the pathogenicity of *M. tb*; for instance, it uses TCSs to control the production of virulent proteins that are vital for survival inside the host. These TCSs signaling transduction systems have structural and pharmacological characteristics that make them vulnerable to inactivation [46] . Owing to the fact that they are essential in *M. tb* infection, they could serve as a promising drug target.

Quorum sensing (QS) is a bacterial communication system that permits specialized activities like biofilm formation, virulence factor production, secondary metabolite synthesis, bioluminescence, and stress adaption mechanisms like bacterial competition systems. *M. tb* drug resistance is associated with a number of mechanisms, one of which is biofilm formation [47] . Thus, focusing on the proteins involved in quorum sensing might benefit in the fight against *M. tb* drug resistance

Druggability and Virulence Factor Analysis of Cytoplasmic Proteins

The druggable analysis of proteins is one of the most critical aspects of the subtractive proteomics strategy. This procedure entails assessing the protein to see if it may be utilised as a therapeutic target. One of the essential characteristics of a suitable drug target protein is its druggability, which indicates whether the protein is susceptible to binding with antagonist compounds. The druggability of a target is generally determined by aligning it with known proteins that have been effectively addressed with drugs in the past [48] . DrugBank database is a complete package of both FDA-approved (4151) and experimental drugs (6654). Moreover,

it has been used in many studies to determine the druggability nature of the proteins [49]. In this study, the druggability of 30 non-homologous essential cytoplasmic proteins has been evaluated using the DrugBank database. A BLASTp search was performed against the DrugBank database's well-established drug targets. The findings revealed that out of 30, only 14 proteins were identified as druggable. The list of the identified druggable proteins along with the name of the drugs and their DrugBank ID is given in Table 1. These 14 druggable targets were further subjected to virulence factor analysis.

Virulence factor analysis identifies those molecules that help the pathogen evade the host defense mechanism. Several pathogenic bacteria including, *Salmonella enterica serovar Typhimurium*[50], *Pseudomonas aeruginosa*[51], *Serratia grimesii* [52], *Serratia proteamaculans* [53], *Staphylococcus epidermidis* [54], *Staphylococcus haemolyticus* [55], *Staphylococcus saprophyticus* [56], *Staphylococcus capitis* [57], *Staphylococcus lugdunensis* [58], etc. produce a wide spectrum of virulent proteins to survive and impair the host defense mechanism during the infection. *M. tb* produces a variety of virulent proteins such as fatty acid/lipid metabolism (e.g., FadD33, Icl1, and Icl2) [59], cell wall proteins (e.g., Erp, Fbp [60], lipoproteins (LppX) [61], secretion system proteins (ESX-1 secretion system) [62], proteases [63], two-component systems (SenX3-RegX3), etc. during its survival in the host. Moreover, many of the *M. tb* virulence genes are conserved in non-pathogenic mycobacteria. VFDB was used to find out the virulent nature of these 14 druggable proteins of *M. tb* CCDC5180. Out of these 14 proteins, 9 proteins were identified to be virulent. The list of the virulent proteins is given in Table 2.

Protein-Protein Interaction Network Analysis of Novel Drug Targets

Protein-protein interactions (PPI) govern a broad spectrum of biological activities, including cell-to-cell interactions, metabolic regulation, and developmental regulation. PPI aid in describing and narrowing down a protein's function, its molecular actions, and its cellular context. The identification of novel therapeutic targets for the treatment of different ailments is aided by the discovery of protein-protein interaction information, which is a major endeavour in fundamental biological research. Several model organism interaction networks (interactomes) have been experimentally developed [64]. However, this huge mass of data is making laboratory validation troublesome. Computational

Table 1: List of non-homologous, essential, and druggable proteins.

Sr no.	Accession Id	Protein name	Drug Name	Drug Group	DrugBank Id
1	AHJ56717.1	Alanine racemase	Cycloserine	Approved	DB00260
2	AHJ54785.1	Anthranilate synthase component I	Formic acid	Experimental, investigational	DB01942
3	AHJ55330.1	ATP phosphoribosyltransferase	1-(5-phospho-D-ribosyl)-ATP	Experimental	DB01661
4	AHJ56227.1	D-alanyl-alanine synthetase A	Cycloserine	Approved	DB00260
5	AHJ56772.1	dTDP-4-dehydrorhamnose 3,5-epimerase	S,S-(2-Hydroxyethyl)Thiocysteine	Experimental	DB04530
6	AHJ54775.1	Histidinol dehydrogenase	Imidazole, Histidinol, 1,4-Dithiothreitol	Experimental, investigational, experimental, experimental	DB03366, DB03811, DB04447
7	AHJ54776.1	Histidinol-phosphate aminotransferase	Pyridoxamine-5'-Phosphate, L-histidinol phosphate	Experimental	DB02142, DB03997
8	AHJ54118.1	Mycobacterial persistence regulator MprA	Guanosine-5'-Monophosphate	Experimental	DB01972
9	AHJ55610.1	Salicylate synthase MbtI	Formic acid	Experimental, investigational	DB01942
10	AHJ56525.1	Two component sensory transduction transcriptional regulatory protein MtrA	Adenosine-5'-Rp-Alpha-Thio-Triphosphate, alpha, beta-Methyleneadenosine 5'-triphosphate, 2-Hydroxyestradiol	Experimental	DB02355, DB02596, DB07706

Table 2: List of proteins involved in the virulence of *M. tb* CCDC5180

Sr no.	Accession Id	Protein name	Pathway
1	AHJ54785.1	Anthranilate synthase component I	Phenylalanine, tyrosine, and tryptophan biosynthesis, Metabolic pathways, Biosynthesis of secondary metabolites, Biosynthesis of amino acids, Quorum sensing
2	AHJ56772.1	dTDP-4-dehydrorhamnose 3,5-epimerase	Streptomycin biosynthesis, Polyketide sugar unit biosynthesis, O-Antigen nucleotide sugar biosynthesis, Metabolic pathways, Biosynthesis of secondary metabolites
3	AHJ54118.1	Mycobacterial persistence regulator MprA	Two-component system
4	AHJ55610.1	Salicylate synthase MbtI	Biosynthesis of siderophore group nonribosomal peptides, Biosynthesis of secondary metabolites
5	AHJ56525.1	Two-component sensory transduction transcriptional regulatory protein MtrA	Two-component system
6	AHJ53595.1	Two-component system sensor protein RegX3	Two-component system
7	AHJ54034.1	Two component system transcriptional regulator PrrA	Two-component system
8	AHJ54175.1	Two component system transcriptional regulator TrcR	Two-component system
9	AHJ54168.1	Transcriptional regulator KdpE	Two-component system, Quorum sensing

evaluation of PPI networks is becoming a necessary strategy for understanding the roles of uncharacterized proteins [65]. One such excellent tool for evaluating PPI networks is the STRING database. STRING contains interactions from a wide range of sources, including known experimental interactions, pathway knowledge from manually curated databases, automated text mining to uncover statistical and semantic links between proteins, and de novo interactions predicted by several algorithms based on genomic data. In the present analysis, the STRING database was used to discern the PPI of three proteins: Two-component system transcriptional regulator (PrrA) and Transcriptional regulator (KdpE).

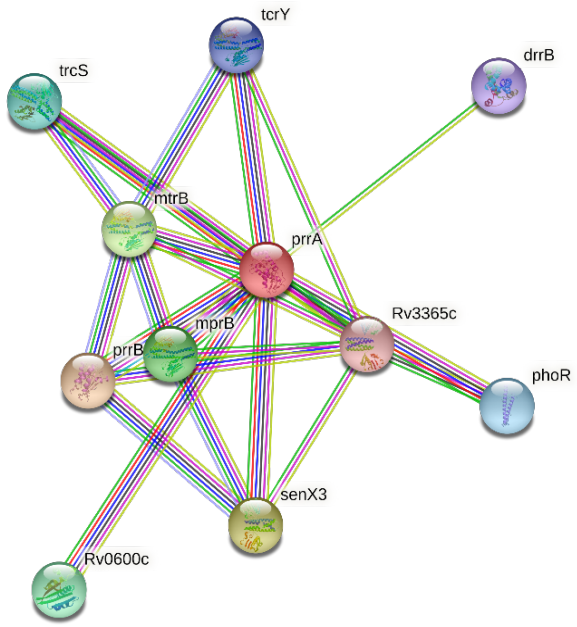

Figure 3: The STRING protein-protein interaction network queried with PrrA. Colored lines between the proteins indicate the various types of interaction.

As shown in Figure 3, PrrA interacts with ten proteins with specificity. These interacting proteins are PrrB, TcrY, DrrB, RV3665c, MtrB, PhoR, SenX3, Rv0600c, MprB, and TrcS. PrrB is a histidine kinase, whereas PrrA is a response regulator, together form the PrrAB system. This system plays a key role in the viability and virulence of *M. tb.* Moreover, they also participate in the early stages of infection [64]. TcrY is also implicated in pathogen virulence , while Parish et al. [66] observed that a mutant strain of *M. tb* lacking tcrY improves pathogen virulence. DrrB gene encodes for Daunorubicin-dim-transport integral membrane protein ABC transporter DrrB. Another ABC transporter protein is Probable dipeptide-transport integral membrane protein ABC transporter DppB, which is a gene product of RV3665c. These proteins are found to be non-essential for the growth of *M. tb*. MtrB, together with MtrA, governs cell division and replication, and their absence results in elongated cells, which indicate a cell division abnormality [66].

PhoR is a response regulator that is required for *M. tb* pathogenicity. The ESX-1 secretion system, whose function is to block essential processes in the host's innate defence mechanisms against pathogens, is controlled indirectly by PhoP and PhoR [46]. SenX3 is an HK that is involved in phosphate sensing. SenX3 and RegX3 are necessary for *M. tb* virulence [67]. Rv0600c, which codes for HKI with an ATP binding domain, is a non-essential protein for *M. tb* growth. MprB regulates ESX-1 function in the same way as PhoR does, and the ESX-1 substrate ESAT-6 is a key virulence factor. TrcS, an HK, is the final interacting protein with PrrA. Unfortunately, there is not much information available on TrcS's function. However, it has been reported that a mutation in TrcR-TrcS causes *M. tb* to become hypervirulent. Therefore, it can be concluded that PrrA interacts with 6 essential proteins that are vital for virulence. Whilst the remaining four proteins are non-essential. Thus, targeting this protein can lead to the attenuation of the infection.

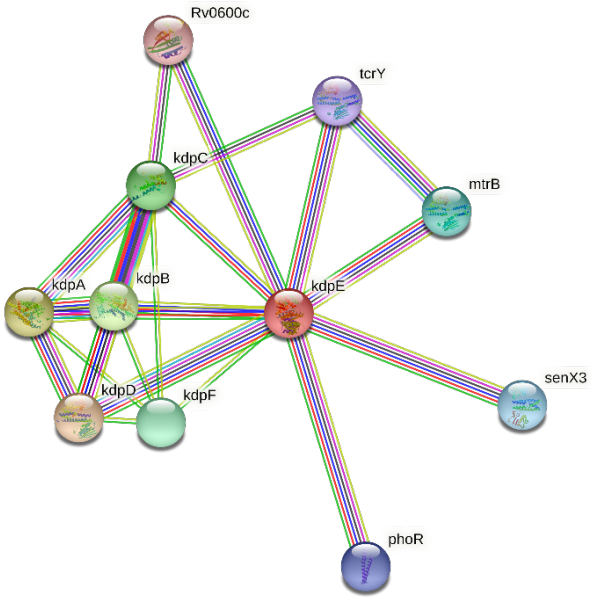

Figure 4: The STRING protein-protein interaction network queried with KdpE. Colored lines between the proteins indicate the various types of interaction.

The next virulent protein is the Transcriptional regulator (KdpE). This protein controls turgor pressure, potassium uptake and also involved in *M. tb* virulence. This TCS protein is also involved in the quorum sensing mechanism [66]. Furthermore, a study conducted by Parish et al. discovered that the deletion of KdpDE resulted in the hypervirulent mutant. [68]The protein-protein interaction analysis of KdpE revealed that it also interacts with 10 proteins (Figure 4). These are TcrY, MtrB, PhoR, SenX3, Rv0600c, KdpD, KdpF, KdpB, KdpA and KdpC. Five of these 10 proteins are found in common between PrrA and KdpE. These five proteins are TcrY, MtrB, PhoR, SenX3, Rv0600c. Except for Rv0600c, all of these proteins are required for *M. tb* pathogenicity. KdpD, on the other hand, is an HK that, together with KdpE, forms *M. tb* TCS and contributes to the organism's pathogenicity. Moreover, it is activated under starvation [50]. The Kdp system consists of

KdpFABC (KdpF, KdpA, KdpB, and KdpC) and KdpDE (KdpD, KdpE). The expression of KdpFABC in *M. tb* is dependent on external K+ concentration. According to Gannoun-Zaki et al., the overexpression of the KdpF gene suppresses *M. bovis* growth in both murine and human primary macrophages [69]. In *E. coli*, the function of KdpFABC is well understood, whereas, in *M. tb*, it is not well comprehended. As a result, more research needs to be done to explore this topic.

Docking Analysis

The molecules investigated in this research belong to a wide variety of phytochemical classes, including alkaloids, flavonoids, and others [26]. Some of the molecules have already been reported as inhibitors of various enzymes involved in tuberculosis infection according to the literature. In this study, we looked at 48 phytochemicals that have previously been identified as anti-tubercular compounds. Molecular docking is one of the most widely used virtual screening methods for predicting the interaction of receptors and ligands. The selected receptors were docked against the screened molecules in the current study to determine binding affinity. The molecules deviating from Lipinski's rule of five were eliminated. The remaining four compounds were selected for each protein. The top four phytochemical molecules, Alianthone, Galbacin, Alloalantolactone, and Cepharanthine, exhibited the lowest binding affinity for the PrrA target and were considered the best. The binding energies of these four prime compounds ranged from -9.0 to -12.1 kcal/mol. For the KdpE target, the four best ligands, Cepharanthine, Ailanthone, Galbacin, and Tuberosin, demonstrated the lowest binding affinity ranging from -9.2 kcal/mol to -10.6 kcal/mol. The docking scores are elaborated in the Table 3.

Table 3: The Docking score of all drugs with their drug target.

Target	Compound	Compound CID	Binding Energy(kcal/mol)
PrrA	Cepharanthine	10206	-12.1
	Alianthone	72965	-9.3
	Galbacin	442873	-9
	Alloalantolactone	474518	-9.3
KdpE	Cepharanthine	10206	-10.6
	Ailanthone	72965	-9.8
	Galbacin	442873	-9.2
	Tuberosin	5318770	-9.7

Cepharanthine and Alianthone had the lowest binding energy for both targets. Cepharanthine (Figure 5 A) had a binding energy of -12.1 kcal/mol and no van der wall bond was observed but a carbon hydrogen bond was formed with ALA130, ASP93, VAL188, and a pi-sigma bond was formed with PRO147. Pi-cation bonding with ARG149 and pi-anion bonding with ASP166 were observed. ARG125 and ARG126 are involved in the formation of an alkyl bond, pi-alkyl bond with ALA169 and Val170. Alianthone, on the other hand, had a binding energy of -9.3 kcal/mol and formed a conventional hydrogen bond with ASP166, SER132, and THR131, as well as a carbon hydrogen bond with PRO62 and ALA130, with zero van der Waals bond.

Cepharanthine, which has a binding energy of -10.6 kcal/mol (Figure 6 A). The alkyl bond was observed at position ALA42, ARG39, LEU38 and ALA35. All of the amino acids associated with the formation of the alky bond interact with the carbon-hydrogen bond at position PRO155. Amino acids that are involved in hydrophobic interactions are ALA35, ALA42 forms (alkyl bond), ARG39 and LEU38 forms (Pi-alkyl bond). Alianthone a binding energy of -9.8 kcal/mol (Figure 6 B), formed four Conventional Hydrogen bonds at GLY66, TRP70, GLU162, PRO155, and two Carbon-Hydrogen bonds at TRP158 and LYS142, as well as two Alkyl bonds at LEU38 and ARG39. Our docking analysis identified Cepharathine and Alianthone as a high-ranking lead compound for developing a tuberculosis drug.

Conclusion

Infection caused by *M. tb*, is one of the leading causes of death worldwide. The ongoing rise in multi and extensive-drug resistance in *M. tb* complicates tuberculosis treatment. The availability of many fully sequenced *M. tb* genomes has allowed investigating the species pangenome as well as its pan-phylogeny to identify potential novel drug targets that will lead to drug discovery. The whole proteome of *M. tb* CCDC5180 was retrieved, the total proteome count was found to be 4160 and out of these 426 essential proteins were selected and 8 proteins were involved in causing virulence. After performing druggability and virulence factor analysis of this cytoplasmic protein two novel target proteins were selected namely PrrA and KdpE which are involved in a

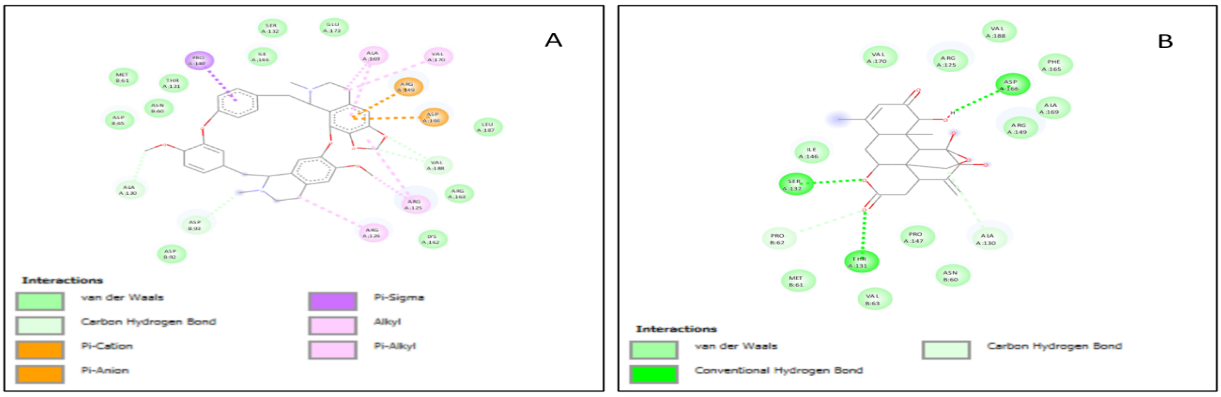

Figure 5: 2D interaction between the protein–ligand complex. Here, figure (**A**) Cepharanthine (**B**) Alianthone showing ligand contact with the protein PrrA after molecular docking

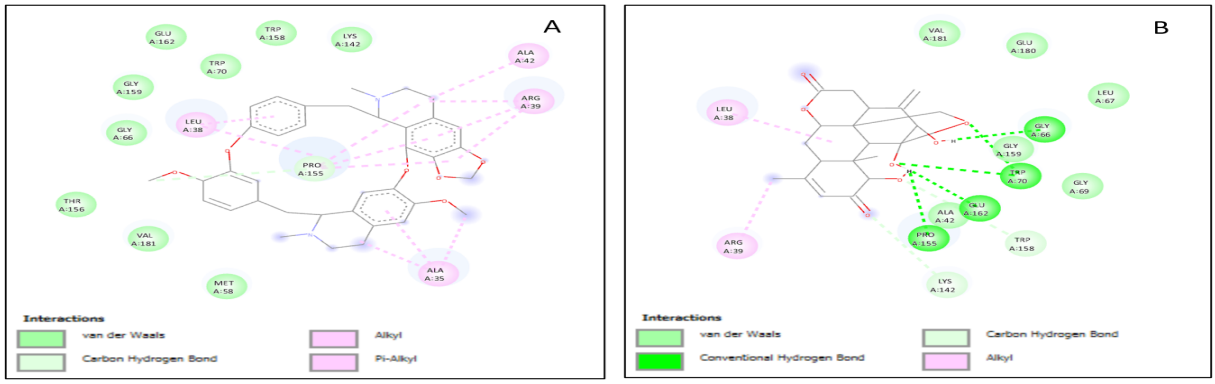

Figure 6*:* 2D interaction between the protein–ligand complex. Here, figure **(A)** Cepharanthine **(B)** Alianthone showing ligand contact with the protein KdpE after molecular docking.

two-component system and crucial for the survival of the *M. tb*. Further by performing molecular docking with 48 compounds, only 2 novel compounds showed good binding affinity towards the target protein PrrA and KdpE. Cepharanthine and Alianthone showed binding energy of -12.1 kcal/mol and -9.3 kcal/mol for PrrA whereas; the binding energies of KdpE were -10.6 kcal/mol and -9.8 kcal/mol, respectively. As a result, these target molecules work against novel target. The findings, we believe, will help in the development of traditional medicine-based therapy methods, as well as the identification of viable hits for future lead optimization in Tuberculosis medication development. The validity of these findings could be strengthened by molecular dynamics simulations of the protein model and experimental studies on animal models, paving the way for the development of powerful-targeted therapies against Mycobacterium infections.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Acknowledgements

We would like to thank Ms. Norine D'Souza and Dr. Karuna Gokarn from St. Xaviers College (Autonomous), Mumbai for valuable guidance and support.

References

1. World Health Organization, Tuberculosis Report, vol. XLIX, 2020; no. 9: 10-11.
2. Cambau E, Drancourt M. Steps towards the discovery of Mycobacterium tuberculosis by Robert Koch, 1882. Clin Microbiol Infect. 2014; 20: 196-201.
3. Pieters J. Mycobacterium tuberculosis and the Macrophage: Maintaining a Balance. Cell Host Microbe. 2008; 3: 399-407.
4. Zaman K. Tuberculosis: A global health problem. J. Heal. Popul. Nutr. 2010; 28: 111-113.
5. McDermott PF, Walker RD, White DG. Antimicrobials: Modes of action and mechanisms of resistance. Int. J. Toxicol. 2003; 22: 135-143.
6. Forrellad MA, Klepp LI, Gioffréet A, et al. Virulence factors of the mycobacterium tuberculosis complex. Virulence. 2013; 4: 3-66.
7. Gengenbacher M, Kaufmann SHE. Mycobacterium tuberculosis: Success through dormancy. FEMS Microbiol Rev. 2012; 36: 514-532.
8. Gygli SM, Borrell S, Trauner A, et al. Antimicrobial resistance in Mycobacterium tuberculosis: Mechanistic and evolutionary perspectives. FEMS Microbiol. Rev. 2017; 41: 354-373.
9. Yuanyuan Zhang, Chen Chen, Jie Liu, et al. Complete genome sequences of Mycobacterium tuberculosis Strains CCDC5079 and CCDC5080, which belong to the Beijing family. J. Bacteriol. 2011; 193; 5591-5592.
10. Lee NH, Lee JA, Park SY, et al. A review of vaccine development and research for industry animals in Korea. Clin Exp Vaccine Res. 2012; 1: 18.
11. Hossain T, Kamruzzaman M, Choudhury TZ, et al. Application of the Subtractive Genomics and Molecular Docking Analysis for the Identification of Novel Putative Drug Targets against Salmonella enterica subsp. enterica serovar Poona. Biomed Res Int. 2017; Article ID 3783714.
12. Uddin R, SufianM. Core proteomic analysis of unique metabolic pathways of salmonella enterica for the identification of potential drug targets. PLoS One. 2016; 11: 1-22.
13. Debmalya Barh, Sandeep Tiwari, Neha Jainet et al. In silico subtractive genomics for target identification in human bacterial pathogens. Drug Dev Res. 2011; 72: 162-177.
14. Francesca Bottacini, Mary O'Connell Motherway, Justin Kuczynski, et al. Comparative genomics of the Bifidobacterium breve taxon. BMC Genomics. 2014; 15: 170.
15. Md. Ismail Hosen, Arif Mohammad Tanmoy, Deena-Al Mahbuba, et al. Application of a subtractive genomics approach for in silico identification and characterization of novel drug targets in Mycobacterium tuberculosis F11. Interdiscip Sci: Comput Life Sci. 2014; 6: 48-56.
16. Zhang R, Ou HY, Zhang CT. DEG: A database of essential genes. Nucleic Acids Res. 2004; 32: 271-272.
17. Huang Y, Niu B, Gao Y, et al. CD-HIT Suite: A web

server for clustering and comparing biological sequences. Bioinformatics. 2010; 26: 680-682.

18. Hizbullah Z, Nazir SG, Afridi M, et al. Reverse vaccinology and subtractive genomics-based putative vaccine targets identification for Burkholderia pseudomallei Bp1651. Microb. Pathog. 2018; 125: 219-229.
19. Gao F, Luo H, Zhang CT, et al. Gene essentiality analysis based on DEG 10, an updated database of essential genes. Methods Mol Biol. 2015; 1279: 219-233.
20. Peng C, Gao F. Protein localization analysis of essential genes in prokaryotes. Sci Rep. 2014; 4: 1-7.
21. Nancy Y. Yu, James R. Wagner, Matthew R. Laird, et al. PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics. 2010; 26: 1608-1615.
22. Ron Caspi, Tomer Altman, Richard Billington, et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. Nucleic Acids Res. 2014; 42: 459-471.
23. David S Wishart, Yannick D Feunang, An C Guo, et al. DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Res. 2018; 46: D1074-D1082.
24. Liu B, Zheng D, Jin Q, et al. VFDB 2019: A comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res. 2019; 47: D687-D692.
25. Damian Szklarczyk, Annika L Gable, David Lyon, et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019; 47: D607-D613.
26. Vikas Jhaa, Sakshi Devkarb, Kunal Gharat, et al. Screening of Phytochemicals as Potential Inhibitors of Breast Cancer using Structure Based Multitargeted Molecular Docking Analysis. Phytomedicine Plus. 2011; 2: 100227-2022.
27. Sen T, Verma NK. Functional annotation and curation of hypothetical proteins present in a newly emerged serotype 1c of Shigella flexneri: Emphasis on selecting targets for virulence and vaccine design studies. Genes. 2020; 11: 340.
28. Barh D, Kumar A. In silico identification of candidate drug and vaccine targets from various pathways in Neisseria gonorrhoeae. In Silico Biol. 2009; 9: 225-231.
29. Azam SS, Shamim A. An insight into the exploration of druggable genome of Streptococcus gordonii for the identification of novel therapeutic candidates. Genomics. 2014; 104: 203-214.
30. Khan MT, Mahmud A, Iqbal A, et al. Subtractive genomics approach towards the identification of novel therapeutic targets against human Bartonella bacilliformis. Informatics Med Unlocked. 2020; 20: 100385.
31. Duffield M, Cooper I, McAlister E. et al. Predicting conserved essential genes in bacteria: In silico identification of putative drug targets. Mol Biosyst. 2010; 6: 2482-2489.
32. Mirzaei Mehrabad E, Hassanzadeh R, Eslahchi C. PMLPR: A novel method for predicting subcellular localization based on recommender systems. Sci Rep. 2018; 8: 1-10.
33. Martínez-Núñez MA, López VELY. Nonribosomal peptides synthetases and their applications in industry. Sustain Chem Process. 2016; 4: 1-8.
34. Natale P, Brüser T, Driessen AJM. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane-Distinct translocases and mechanisms. Biochim Biophys Acta Biomembr. 2008; 1778: 1735-1756.
35. Pierre LeMagueres, Hookang Im, Jerry Ebalunode, et al. The 1.9 Å crystal structure of alanine racemase from Mycobacterium tuberculosis contains a conserved entryway into the active site. Biochemistry. 2005; 44: 1471-1481.
36. Gillner DM, Becker DP, Holz RC. Lysine biosynthesis in bacteria: A metallodesuccinylase as a potential antimicrobial target. J. Biol. Inorg. Chem. 2013; 18: 155-163.
37. Consalvi S, Scarpecci C, Biava M, et al. Mycobacterial tryptophan biosynthesis: A promising target for tuberculosis drug development? Bioorganic Med Chem Lett. 2019; 29: 126731.
38. ichi Kimura K. Liposidomycin, the first reported nucleoside antibiotic inhibitor of peptidoglycan biosynthesis translocase I: The discovery of liposidomycin and related compounds with a perspective on their application to new antibiotics. J. Antibiot. 2019; 72: 877-889.
39. Marrakchi H, Lanéelle MA, Daffé M. Mycolic acids: Structures, biosynthesis, and beyond. Chem Biol. 2014; 21: 67-85.
40. Dulberger CL, Rubin EJ, Boutte CC. The mycobacterial cell envelope — a moving target. Nat Rev Microbiol. 2020; 18: 47-59.
41. Nigou J, Gilleron M, Puzo G. Lipoarabinomannans: From structure to biosynthesis. Biochimie. 2003; 85: 153-166.
42. Weissman KJ, Leadlay PF. Combinatorial biosynthesis of reduced polyketides. Nat Rev Microbiol. 2005; 3: 925-936.
43. Ma Y, Pan F, McNeil M. Formation of dTDP-rhamnose is essential for growth of mycobacteria. J Bacteriol. 2002; 184: 3392-3395.
44. Alderwick LJ, Harrison J, Lloyd GS, et al. T 1969; 1–15, 2015.
45. Reyes AG, Godínez O, Mejía A. Importance of two component systems regulation in Streptomyces strains. Rom Biotechnol Lett. 2017; 22: 1-10.
46. Srijon Kaushik Banerjee, Manish Kumar, Reshma Alokam, et al. Targeting multiple response regulators of Mycobacterium tuberculosis augments the host immune response to infection. Sci Rep. 2016; 6: 1-15.
47. Rocio Trastoy Pena, Lucia Blasco, Antón Ambroa, et al. Relationship between quorum sensing and secretion systems. Front. Microbiol. 2019; 10: 1100.
48. Islam MS, Richards JP, Ojha Ak. Targeting drug tolerance

in mycobacteria: A perspective from mycobacterial biofilms. Expert Rev Anti Infect Ther. 2012; 10: 1055-1066, 2012.

49. Ghattas MA, Raslan N, Sadeq A, et al. Druggability analysis and classification of protein tyrosine phosphatase active sites. Drug Des Devel Ther. 2016; 10: 3197-3209.
50. Abdur Rehman, Xiukang Wang, Sajjad Ahmad, et al. In silico core proteomics and molecular docking approaches for the identification of novel inhibitors against streptococcus pyogenes. Int J Environ Res Public Health. 2021; 18: 21. 11355.
51. Thaís Cristina Vilela Rodrigues, Arun Kumar Jaiswal, Alissa de Sarom, et al. Reverse vaccinology and subtractive genomics reveal new therapeutic targets against Mycoplasma pneumoniae: A causative agent of pneumonia. R Soc Open Sci. 2019; 6: 190907.
52. Yang X, Wang J, Feng Z, et al. Relation of the pdxB-usg-truA-deda Operon and the truA gene to the intracellular survival of Salmonella enterica serovar typhimurium. Int J Mol Sci. 2019; 20: 380.
53. Justyna Roszkowiak, Paweł Jajor, Grzegorz Guła, et al. Interspecies outer membrane vesicles (OMVs) modulate the sensitivity of pathogenic bacteria and pathogenic yeasts to cationic peptides and serum complement. Int J Mol Sci. 2019; 20: 5577.
54. Khaitlina S, Bozhokina E, Tsaplina O, et al. Bacterial actin-specific endoproteases grimelysin and protealysin as virulence factors contributing to the invasive activities of Serratia. Int J Mol Sci. 2020; 21: 4025.
55. Steven R Gill, Derrick E Fouts, Gordon L Archer, et al. Insights on Evolution of Virulence and Resistance from the Complete Genome Analysis of an Early Methicillin-Resistant. J Bacteriol. 2005; 187: 2426-2438.
56. Barros Em, Ceotto H, Bastos MCF, et al. Staphylococcus haemolyticus as an important hospital pathogen and carrier of methicillin resistance genes. J Clin Microbiol. 2012; 50: 166-168.
57. de Paiva-Santos W, de Sousa VS, Giambiagi-deMarval M. Occurrence of virulence-associated genes among Staphylococcus saprophyticus isolated from different sources. Microb Pathog. 2018; 119: 9-11.
58. Wil C Van Der Zwet, Yvette J Debets-Ossenkopp, Erik Reinders, et al. Nosocomial spread of a Staphylococcus capitis strain with heteroresistance to vancomycin in a neonatal intensive care unit. J Clin Microbiol. 2002; 40: 2520-2525.
59. Argemi X, Hansmann Y, Prola K, et al. Coagulase-negative staphylococci pathogenomics. Int J Mol Sci. 2019; 20: 1-19.
60. Manabe YC, Saviola BJ, Sun L. et al. Attenuation of virulence in Mycobacterium tuberculosis expressing a constitutively active iron repressor. Proc Natl Acad Sci. 1999; 96: 12844-12848.
61. Alemayehu Godana Birhanu, Solomon Abebe Yimer, Shewit Kalayou, et al. Ample glycosylation in membrane and cell envelope proteins may explain the phenotypic diversity and virulence in the Mycobacterium tuberculosis complex. Sci Rep. 2019; 9: 1-15.
62. Gerlind Sulzenbache, Stéphane Canaan, Yann Bordat, et al. LppX is a lipoprotein required for the translocation of phthiocerol dimycocerosates to the surface of Mycobacterium tuberculosis. EMBO J. 2006; 25: 1436-1444.
63. Abdallah M. Abdallah, Nicolaas C. Gey van Pittius, Patricia A. DiGiuseppe Champion, et al. Type VII secretion - Mycobacteria show the way. Nat Rev Microbiol. 2007; 5: 883-891.
64. Hamza Arshad Dar, Tahreem Zaheer, Nimat Ullah, et al. Pangenome analysis of mycobacterium tuberculosis reveals core-drug targets and screening of promising lead compounds for drug discovery. Antibiotics. 2020; 9: 1-14.
65. Ren JX, Qian HL, Huang YX, et al. Virtual screening for the identification of novel inhibitors of Mycobacterium tuberculosis cell wall synthesis: Inhibitors targeting RmlB and RmlC. Comput Biol Med. 2015; 58: 110-117.
66. Elena Pini, Giulio Poli, Tiziano Tuccinardi, et al. New chromane-based derivatives as inhibitors of mycobacterium tuberculosis salicylate synthase (mbti): Preliminary biological evaluation and molecular modeling studies. Molecules. 2018; 23: 1506.
67. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993; 10: 512-526.
68. Parish T. Two-component regulatory systems of mycobacteria. Mol Genet Mycobact. 2015; 2: MGM2-0010-2013.
69. Gannoun-Zaki L, Alibaud L, Carrère-Kremer S, et al. Over-expression of the KdpF Membrane Peptide in Mycobacterium bovis BCG Results in Reduced Intramacrophage Growth and Altered Cording Morphology. PLoS One. 2013; 8: e60379.