Bioinformatic Exploration of Metal-Binding Proteome of Zoonotic Pathogen Orientia tsutsugamushi

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Metal ions are involved in many essential biological processes and are crucial for the survival of all organisms. Identification of metal-binding proteins (MBPs) of human affecting pathogens may provide the blueprint for understanding biological metal usage and their putative roles in pathogenesis. This study is focused on the analysis of MBPs from Orientia tsutsugamushi (Ott), a causal agent of scrub typhus in humans. A total of 321 proteins were predicted as putative MBPs, based on sequence search and three-dimensional structure analysis. Majority of proteins could bind with magnesium, and the order of metal binding was Mg > Ca > Zn > Mn > Fe > Cd > Ni > Co > Cu, respectively. The predicted MBPs were functionally classified into nine broad classes. Among them, gene expression and regulation, metabolism, cell signaling, and transport classes were dominant. It was noted that the putative MBPs were localized in all subcellular compartments of Ott, but majorly found in the cytoplasm. Additionally, it was revealed that out of 321 predicted MBPs 245 proteins were putative bacterial toxins and among them, 98 proteins were nonhomologous to human proteome. Sixty putative MBPs showed the ability to interact with drug or drug-like molecules, which indicate that they may be used as broad-spectrum drug targets. These predicted MBPs from Ott could play vital role(s) in various cellular activities and virulence, hence may serve as plausible therapeutic targets to design metal-based drugs to curtail its infection.

Keywords: Orientia tsutsugamushi, metal-binding proteins, therapeutic targets, structure modeling, bioinformatics

INTRODUCTION

Orientia tsutsugamushi (Ott) is a Gram-negative mite-borne bacterium responsible for life-threatening zoonotic disease, scrub typhus (Walker, 2016; Jain et al., 2018; Jiang et al., 2018). Scrub typhus (mite-borne typhus) represents one of the oldest vector-borne disease which is endemic in Asia Pacific, with some reports from Middle East and South America. One billion people are at risk of attaining the infection and one million people get infected each year globally (Xu et al., 2017). The outbreak of the infection was reported from different Indian provinces and is related to various complications with 30% fatality rate or even higher (Mahajan et al., 2010; Khan et al., 2017; Xu et al., 2017; Jain et al., 2018). There are certain antibiotics for the treatment of disease, but resistance to these antibiotics by strains of Ott has been reported and reviewed earlier (Watt et al., 1996; Mathai et al., 2003; Kelly et al., 2017). According
to the Centers for Disease Control and Prevention of the United States of America, there is no effective licensed vaccine available for scrub typhus till date.

The ancient bacteria have originated from metal-rich environments, and therefore, metal ions (mostly transition metals) are integral constituents of proteins (Maret, 2016). Metal ions, such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) are engaged in vital biological processes and are crucial for the survival of the microorganisms. Approximately 45% of the proteins require metal ion as a cofactor for their functioning (Klein and Lewinson, 2011). The bacterial pathogens have the ability to sense metal ions, which is important to invade the host tissue and cause disease. The bacterial metalloproteases may disrupt the important host physiological processes such as destructing key signaling intermediates, breakdown of barriers, and release of metals from the host metalloproteins (Porcheron et al., 2013; Ma et al., 2015).

The coevolution of pathogens and host for the desire of metal ions as an indispensable component of cellular metabolism revealed emerging paradigms in the field of microbiology, rapid evolution, and metal homeostasis (Palmer and Skaar, 2016). Therefore, to acquire a deep understanding of metal homeostasis mechanisms of the pathogens, we first need detailed knowledge of their metal-binding proteins (MBPs). The computational biology and bioinformatics have emerged as highly promising, rapid and efficient approaches for extracting information of genes and proteins from the available sequence data for commercial and therapeutic applications (Singh et al., 2017; Sharma et al., 2018a). In addition, the in silico approaches are time saving, less expensive, and can serve as the startup for further experimental studies. We herein present the detailed in silico report on metalloproteome of human bacterial pathogen, Orientia tsutsugamushi describing its putative MBPs that are probably involved in virulence and may serve as potential targets for the drug discovery process.

MATERIALS AND METHODS

Sequence Data Retrieval

The available complete dataset of protein sequences of Orientia tsutsugamushi strain Ikeda (Nakayama et al., 2008) was downloaded from the RefSeq database at the National Centre for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/010/205/GCF_000010205.1_ASM1020v1/GCF_000010205.1_ASM1020v1_protein.faa.gz). RefSeq is the comprehensive, curated, annotated, and nonredundant sequence database of genomes, transcripts, and proteins (Pruitt et al., 2006).

Identification of Putative MBPs of Orientia tsutsugamushi

The overall scheme of the work is explained in Figure 1. The prediction of MBPs was carried out in two steps. In the first step, the search for MBPs was performed in Uniprot. The keywords (iron binding, zinc binding, calcium binding, magnesium binding, manganese binding, copper binding, cadmium binding, cobalt binding, and nickel binding) were used as inputs to identify proteins which bind to respective metals. The resulting data were retrieved from Uniprot protein knowledgebase (release November 2017). The retrieved data were converted into local database, and standalone BLASTp (Altschul et al., 1990) search for Mt proteome was performed with expect value (E-value) 0.00001. This was performed in order to acquire the authentic sequences with considerable similarity to other annotated MBPs in the database. In the second step, the shortlisted proteins from the first step were searched against MetalPDB database with E-value of 0.00001. MetalPDB is the database which offers the information on various metal-binding sites present in three-dimensional (3D) structure of biological macromolecules (Putignano et al., 2017). The proteins which showed homology with desired E-value cutoff (≤0.00001) were nominated as putative MBPs of Orientia tsutsugamushi.

Three-Dimensional Structure Modeling and Metal Docking

3D structure modeling of the putative MBPs of Orientia tsutsugamushi was performed by Protein Homology/analogy Recognition Engine v2.0 (Phyre2) program (Kelley et al., 2015). The criteria was set as earlier defined, i.e., confidence score of ≥90% and coverage ≥50% (Sharma et al., 2017). The Phyre2 tool employs the Hidden Markov Model (HMM-HMM) alignment homology approach to predict and shape 3D protein structures. The 3D modeled proteins were further investigated for metal (Fe2+, Fe3+, Zn2+, Cu2+, Cu2+, Mg2+, Mn2+, Ca2+, Cd2+, Ni2+, and Co2+) binding structural motifs with metal ion-binding site prediction and docking server (MIB) (Lin et al., 2016). MIB uses the fragment transformation method to predict the residues which bind to the metal ion within the range of 3.5Å (Lin et al., 2016).

Subcellular Localization

The subcellular localization of the predicted metalloproteins was carried out by Gneg-mPLoc (Shen and Chou, 2010), CELLO (Yu et al., 2004), and PSORTb 3.0.2 (Yu et al., 2010). Gneg-mPLoc is based on the information of functional domain, Gene Ontology, and sequential evolution. CELLO uses support vector machine classifiers, which are based on numerous n-peptide compositions to predict the subcellular location of the Gram-negative bacterial proteins. PSORTb employs six modules and creates a Bayesian network for predicting the final subcellular localization on the basis of performance of each module. These tools are specific and efficient for the prediction of subcellular location of Gram-negative bacterial proteins, and consensus of these tools was used for final prediction. The overall success rates accomplished by these bioinformatics servers, i.e., Gneg-mPLoc, CELLO, and PSORTb 3.0.2, are 85.5%, 89%, and 98.3%, respectively.

Functional Classification and Gene Ontology Network Construction of Putative MBPs

The functional annotation of the putative MBPs was done by exploring conserved domains, family and superfamilies with the help of InterProScan (Jones et al., 2014) and Pfam (Finn et al., 2015) at
default parameters. The literature survey was performed for each predicted MBP based on the identified domain/family, and then, these proteins were further divided into broad functional classes. The predicted MBPs of Ott were clustered on the basis of identified domains using MEGA software, ver. 6 (Tamura et al., 2013). The constructed clustergram was further visualized by EvolView v2 program (He et al., 2016). After domain-based functional annotation, the Gene Ontology (GO) functional (biological and molecular) network construction of putative MBPs was executed by ClueGO v2.3.3 (Bindea et al., 2009) plugin of Cytoscape (Shannon et al., 2003). Each GO biological process or GO molecular function term was depicted by node (circle), and the contacts between GO terms (biological or molecular) were represented by edge. ClueGO utilizes kappa score (statistical method) for functional grouping of the GO terms (Huang et al., 2007), and significance of the network was determined by node size.

**Bacterial Toxins Prediction**

The bacterial toxins are considered as one of the effective targets for drug development process. We used the Database of Bacterial Exotoxins for Humans (DBETH) (Chakraborty et al., 2011) database for prediction of virulence factors. DBETH database utilizes support vector machine algorithm to predict bacterial toxins of human affecting pathogens. We have first performed the DBETH analysis on the whole proteome of Ott strain Ikeda and then extracted putative virulent MBPs among them. This was done in order to check the proportion of putative virulent MBPs among the total putative virulent proteins of Ott strain Ikeda.

**Subtractive Proteomic Approach**

Furthermore, to avoid cytotoxicity and cross-reactivity of the drug with the host cell, we have adopted the subtractive proteomics approach.
approach. Using this approach, the BLASTp search was performed on screened putative virulent MBPs (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) against the host (human; taxid: 9906) proteome at E-value of 0.0001 in order to select the nonhomologous proteins and to exclude homologous proteins. The putative virulent MBPs showing hits at E-value ≤10^{-4} were considered as homologous and were neglected from the further study. The rest of putative virulent nonhomologous proteins were shortlisted for further analysis.

**Druggability Analysis**

Furthermore, to select suitable or efficient therapeutic targets the physiochemical characterization and druggability analysis of the shortlisted putative virulent nonhomologous MBPs were performed. The Expasy's ProtParam (Gasteiger et al., 2003) was used for the physiochemical characterization. The physiochemical parameters [molecular weight, theoretical PI, aliphatic index, instability index, and grand average of the hydropathicity (GRAVY)] estimation provides deep insight into biochemical behavior of the protein, which can serve as the basis for further studies like developing drugs against pathogens. DrugBank Version 5.0.11 (Wishart et al., 2017) was used to evaluate the druggable properties of putative virulent MBPs. The search of predicted virulent nonhomologous MBPs was performed against the DrugBank database at 0.00001 E-value, and other parameters were set as default. DrugBank is the freely accessible comprehensive database containing detailed information about drug targets and drug data. It contains nonredundant protein (i.e., drug targets) sequences which are linked to the drug entries in the database. The protein showing homology with the DrugBank database was appraised as probable druggable proteins or druggable targets.

**RESULTS AND DISCUSSION**

The report presents in silico proteome-wide identification of putative MBPs of human intracellular bacterial pathogen Ott. Notably, numerous metal ions are essential for microorganisms and play vital role(s) ranging from acting as a cofactor for enzymes, DNA replication, oxidative stress, structural stability, and bacterial virulence (Porcheron et al., 2013; Ma et al., 2015; Palmer and Skaar, 2016). Ott is an intracellular pathogen causing scrub typhus with vast incidents of infections across the globe. In this study, we have integrated various standard computational tools to investigate the putative MBPs in Ott. Nearly 24.22% of the total proteins were the putative MBPs, which conform to earlier reports revealing that one quarter of the bacterial proteins require metals for their biological activities (Waldron and Robinson, 2009). The in silico mining of MBPs of an organism provides primarily information on size, nature, and functional diversity of its metalloproteome. Furthermore, the putatively identified MBPs probably serve as viable targets for experimental studies, which may enhance our knowledge regarding metal homeostasis mechanism of the particular organism. Earlier, the proteome scale in silico approaches has been efficiently implemented for the prediction and characterization of MBPs in plant pathogens (Sharma et al., 2017; Sharma et al., 2018a; Sharma et al., 2019).

Bacteria exploit a variety of metal acquisition and export system to overcome the host defense strategies and to maintain the metal homeostasis by various transcriptional regulators, which help them to adapt the changing environmental conditions (Porcheron et al., 2013). Indeed, to curtail pathogen outgrowth, host employs different defense strategies like metal starvation to bacteria by metal sequestration mechanism (nutritional immunity) and metal toxicity by the release of metal ions in high concentration (Palmer and Skaar, 2016). The necessity of these metal ions during pathogenesis is because of their involvement in various cellular processes. Iron is a vital micronutrient which acts as a cofactor for various enzymes and play important role(s) in various metabolic processes, i.e., energy generation, tricarboxylic acid (TCA) cycle, DNA replication, and protection against oxidative stress (Skaar, 2010; Ma et al., 2015). Bacteria secrete siderophores to acquire iron from the host cell and regulate iron-mediated virulence (Skaar, 2010). Zinc is also a crucial micronutrient involved in structural stability, catalytic activities, and regulation of vital biological processes including mechanisms of virulence such as invasion, formation of biofilm, and adhesion to host cells (Shafeeq et al., 2013). The role of Mg^{2+} (most abundant divalent metal ion in a living cell) is also well known earlier in numerous physiological processes, transport, and virulence of bacteria (Groisman et al., 2013). The function of Ca^{2+} in bacteria is mostly noticed in protein stability, cell signaling, cell cycle, and cell division (Michiels et al., 2002). Other metals such as Cu, Cd, Mn, Co, and Ni integrate with biomolecules such as proteins and play catalytic role(s) in various cellular and biological process ranging from respiration, DNA replication, transcription, and response to oxidative stress (Kim et al., 2008; Porcheron et al., 2013; Palmer and Skaar, 2016).

**Predicted MBPs of Orientia tsutsugamushi and Their Metal-Binding Patterns**

The standalone BLASTp search of Ott whole proteome (1,325 proteins) was accomplished with the dataset of MBPs that was retrieved from the Uniprot. The resultant protein sequences which specify the defined threshold were further selected for second step. In the second step, the selected protein sequences were subjected to MetalPDB search. A total of 605 proteins were shortlisted further, which followed the desired cutoff. It was found that many protein sequences could bind with more than one metal; therefore, the shortlisted 605 proteins were checked for their ability to bind with more than one metal. A total of 345 proteins could bind with one or more than one metal and considered as putative MBPs (Table S1).

The regions of similarity are located by the alignment between two proteins, which are useful to fetch functional, structural, and evolutionary information (Rost, 1999). Identification of sequence similarity of anonymous protein with the validated proteins of known function submitted in the database enables homology-based annotation (Friedberg, 2006; Singh et al., 2017). In our study, BLASTp search with Uniprot MBPs dataset and MetalPDB database predicted that Ott metalloproteome is predominantly
Distribution of Putative MBPs in Bacterial Cell

The distribution of MBPs in the subcellular compartments of Ott was investigated. Consensus of three prediction programs was used in the study, i.e., Gneg-mPLoc, CELLO, and PSORTb 3.0. The subcellular localization of the 321 MBPs of Ott showed that rich in Mg followed by Ca, Zn, Mn, Fe, Cd, Ni, Co, and Cu (Figure 2). The presence of fewer fractions of Cu-binding proteins correlates towards copper’s efficiency in high redox activity that leads to generation of reactive oxygen species, oxidative stress, and copper toxicity, ultimately damaging the bacterial cell (Andreini et al., 2007b). Therefore, lower concentration of intracellular Cu is needed to be maintained efficiently to prevent toxicity in the cell.

The 3D-structure modeling of the identified 345 putative MBPs was performed by Phyre2. Among these putative MBPs of Ott, 325 proteins were modeled by homology modeling method within the specified criteria of query coverage ≥50 and confidence ≥90. The 3D structure of the protein aid in understanding its function and also provides insight into their molecular mechanism (Ilgü et al., 2016). It was investigated with MIB that 321 proteins had structure motifs for binding different metal ions. These 321 proteins were considered as putative MBPs of Ott, which had both sequence and structural motifs for binding metal ions (Table 1). The obtained putative MBPs showed diversity in their metal-binding sites. The common interacting amino acid residues within binding pocket for Mg2+ ion was Asp, Ser, Lys, and Glu; for Ca2+ was Asp, Glu, and Asn; for Zn2+ was Cys, His, Asp, and Glu; for Mn2+ was Glu, Asp, and His; for Fe3+ was Asp, Glu, and His; for Fe2+ was Glu, Cys, His, and Asp; for Cd2+ was Glu, Gln, and His; for Ni2+ His, Glu, and Asp; for Co3+ was Glu, His, and Asp; for Cu2+ was His and Cys; and for Cu+ was His and Asp. The pattern of interacting amino acid residues with different metal ions are shown in Figure 3, and details of interacting residues are listed in Tables S2A–S2K. Our findings conform to earlier reports showing that charged amino acid residues are required for the coordination of metal ions (Liu et al., 2012; Akcapinar and Sezerman, 2017).

**Table 1** Matrix representing Mg-, Ca-, Zn-, Mn-, Fe-, Cd-, Ni-, Co-, and Cu-binding proteins.

| Metals | Mg | Ca | Zn | Mn | Fe | Cd | Ni | Co | Cu |
|--------|----|----|----|----|----|----|----|----|----|
| Mg     | 193| 71 | 40 | 27 | 22 | 11 | 12 | 8  | 3  |
| Ca     | 71 | 111| 23 | 22 | 8  | 13 | 4  | 4  | 2  |
| Zn     | 40 | 23 | 76 | 16 | 7  | 2  | 5  | 1  |    |
| Mn     | 27 | 22 | 16 | 65 | 6  | 3  | 4  | 7  | 0  |
| Fe     | 22 | 8  | 7  | 6  | 52 | 4  | 1  | 3  | 2  |
| Cd     | 11 | 13 | 2  | 3  | 4  | 24 | 4  | 0  | 1  |
| Ni     | 12 | 4  | 2  | 4  | 1  | 4  | 17 | 1  | 0  |
| Co     | 8  | 4  | 5  | 7  | 3  | 0  | 1  | 13 | 0  |
| Cu     | 3  | 2  | 1  | 0  | 2  | 1  | 0  | 8  |    |

A considerable amount of MBPs were observed in the cytoplasm, which encompasses three-fourths of the identified MBPs. The highest number of Mg binding (154 proteins) found in cytoplasm, followed by Ca binding (88 proteins), Zn binding (64 proteins), Mn binding (59 proteins), Fe binding (39 proteins), Co binding (13 proteins), equal number of Cd and Ni binding (10 proteins), and Cu binding (5 proteins). It is reviewed earlier that, to meet the cellular metal demand, the cytoplasm should efficiently concentrate metal ions (Ma et al., 2009). Furthermore, the proteome scale subcellular compartmentalization of other intracellular bacteria like *Rickettsia typhi*, *Leptospira interrogans*, and *Mycobacterium tuberculosis* (*M. tuberculosis*) also suggest that most of the proteins localized in cytoplasm (Viratyosin et al., 2008; Sears et al., 2012; Zhu et al., 2015).

In the current study, Mg-binding proteins were found in four subcellular locations, i.e., cytoplasm, inner membrane, outer membrane, and extracellular with a highest fraction (154 Mg-binding sequences) in the cytoplasm. The extensive presence of Mg-binding proteins in cytoplasm might be interpreted either for stabilizing and neutralizing nucleic acids (DNA and RNA) or probably in ribosome assembling (Groisman et al., 2013; Mushegian, 2016). Calcium accounted to be the second abundant metal in the study and the pivotal role of Ca in protein stability, signal transducer in cell cycle, and cell division observed from the existence of 88 Ca-binding proteins present in cytoplasm (Michiels et al., 2002). Zn-binding proteins were found in all the compartments of the bacterial cell, with 64 proteins being in the cell cytoplasm. The ample use of Zn in the cytoplasm of bacteria may be for the regulation of gene expression and for maintaining integrity of the genome (Bertini et al., 2010; Porcheron et al., 2013). Approximate amount of Fe- (11 proteins) and Zn-binding proteins (9 proteins) were detected in the inner membrane. The occurrence of Fe-binding proteins in the inner membrane explains their notable role of...
electron transport in electron transport chain (Andreini et al., 2007a). Zn-binding proteins in the inner membrane might be involved in import and export of nutrients, metal ions, and toxic substances (Ma et al., 2015). The presence of three Cu-binding proteins in the inner membrane defines their significant role as cofactor of cytochrome c oxidase (Samanovic et al., 2012). A small fraction of MBPs are found to present in the outer membrane, which can help to promote bacterial invasion and transfer of substances across bacterial cell (El-Housseiny et al., 2010; Rollauer et al., 2015). The findings have proclaimed that subcellular localization of predicted MBPs provided the basis for functional information.

**Functional Classification of Putative MBPs**

The functional annotation of predicted MBPs showed that proteins containing ankyrin repeat (23), HD domain (20), DnaB-like helicase (17), ABC transporter (12), and tetratricopeptide repeat (TPR) (11) were predominantly present (Table S3A). The domain prediction of MBPs and their literature study enables us to identify their function and classify them into nine broad functional classes, i.e., gene expression and regulation (127 proteins), metabolism (84 proteins), cell signaling (53 proteins), transport (26 proteins), posttranslational modification (9 proteins), protein folding (9 proteins), stress response regulator (5 proteins), proteolysis (4 proteins), and antimicrobial resistance (4 proteins) (Figure 4, Table S3A). Earlier, Cho et al. (2010) used microarray and proteomic approaches to study global gene expression of Ott strain Boryong and stated that most of the expressed genes belong to the functional classes protein translation, protein processing or secretion, and DNA repair/replication (Cho et al., 2010). This study also supported our findings as we have also noticed that most of the proteins found in the category of gene expression and regulation.

Furthermore, the whole genome sequencing of the strains of Ott revealed that genomes contain repetitive sequences and many of them were pseudogenes (Nakayama et al., 2008; Batty et al., 2018). Ankyrin repeat-containing proteins (Anks), TPR, HD domains, and DnaB helicase are some families of repeats which belong to the category of pseudogenes. In our study, we have noticed the presence of these families, which indicate that metalloproteome of Ott also contain pseudogenes. In addition to Orientia, other obligate and facultative intracellular bacteria (Coxiella burnetii, Rickettsia spp., L. pneumophila,
Wolbachia pipientis, and Anaplasma phagocytophilum) also contain ankyrin repeats (Voth et al., 2009), and these repeats are functionally diverse. A report on Anks of Ott strain Ikeda revealed that Anks resemble with substrates of type-1 secretion system, which inserts traffic to different subcellular localizations or shows a tropism for secretory pathways of the host cell and modulates host cell processes during the infection (VieBrock et al., 2015). Beyer et al. (2015) reported that Anks of Ott strain Ikeda have a eukaryotic/Pox-virus F-box motif which recruits or co-opt host cell SCF1 polyubiquitination machinery and exploit host cell ubiquitination (Beyer et al., 2015). Furthermore, regarding metal-binding activity of Anks, it was reported previously that some Anks require cations for their structural and functional stability (Campanacci et al., 2013). Recently, an in vitro study on TPR proteins of Ott indicate that these binds to DDX3 RNA helicases through N-terminal of DEAD box domain to inhibit eukaryotic translation and to enhance their own replication (Bang et al., 2016). HD domain containing proteins is one of the dominating categories in the metalloproteome of Orientia. The finding is supported by earlier studies that HD domain defines metal-dependent phosphohydrolases, i.e., require different metal ions for their catalytic activity (Aravind and Koonin, 1998; Huynh et al., 2015). Furthermore, a report on HD domain phosphodiesterase of intracellular pathogen Listeria monocytogenes indicates that this domain is involved in cooperative hydrolysis of c-di-AMP and affects bacterial growth, physiology, and virulence (Huynh et al., 2015). Replicative DnaB helicases also reported in our study, which requires divalent cations for their activity and catalyzes the separation of double-stranded DNA into single-stranded DNA in an ATP-dependent manner (Son et al., 2003). Our report also enriched with ABC transporters which are conserved across all the organisms (Higgins, 1992). Furthermore, it is documented earlier that microbial ABC transporters are involved in import and export of wide range of substrates (metals ions and their ionic complexes, amino acids, metabolites, sugars, lipids, and antibiotics) and play
indispensable roles in their growth, survival, and pathogenesis (Garmory and Titball, 2004; Renesto et al., 2005).

The functional annotation of Zn-binding proteins showed the appearance of ABC transporters, metallo-β-lactamase, zinc finger, and DnaJ domain. The identified domains of Zn-binding proteins have important roles in transporting ions, nutrients and toxic substances, gene regulation, antimicrobial resistance, and protein folding (Wommer et al., 2002; Bertini et al., 2010). The chief domains found in Fe-binding proteins of Ott are cytochrome, ABC transporters, ankyrin repeats, glutaredoxin, cysteine desulfurase, and succinate dehydrogenase. These Fe-binding domains may be involved in a variety of functions ranging from metabolism, transport, gene regulation to posttranslational modification (Palmer and Skaar, 2016; Sharma et al., 2018a). The Fe transport and concentration helps in regulating growth and metabolism of Gram-negative bacteria (Kim et al., 2009).

The predicted Mn-binding proteins mainly showed the predominant presence of HD domain, which is primarily involved in signal transduction and metabolism. The role of HD domain has already been discussed in the above paragraph. Predicted 17 Ni-binding proteins mainly comprised of ABC transporters, which may be involved in the transport of toxic substances and metal ions. Earlier, it was reported that Ni transport and metabolism allows immense colonization of Helicobacter pylori and Staphylococcus aureus (Benoit et al., 2013; Remy et al., 2013). In Co-binding proteins, metalloproteinase (M24 and M20) and ribonucleotide reductase are the prime domains which might help in metabolic process and mediates the synthesis of precursor for DNA replication, respectively (Rawlings and Barrett, 1995; Torreans, 2014). It was also documented previously that Ni- and Co-binding proteins are involved in gene expression regulation and protein metabolism (Sun et al., 2013). The computationally predicted Cu-binding proteins of Ott are less in number (0.6% of total proteome) than other predicted MBPs. The results were in accordance with the earlier reports that the fraction of Cu-binding proteins in prokaryotes is <1% (Andreini et al., 2007b). Cytochrome c, CuA, and copper chaperone were the main domains present in identified Cu-binding proteins. The literature survey showed that these Cu-binding proteins play a significant role in metabolic process, stress response, and protein folding (Festa and Thiele, 2011; Palm-Espling et al., 2012).

The predicted Cd-binding proteins constitute up to 1.81% of the total proteome, with typically Zn-/Mg-/Ca-/Fe-/Mn-/Ni-binding motifs. The observation is in accordance with the earlier studies that nonessential heavy metal ion Cd\(^{2+}\) has the ability to replace native metal ions in metalloproteins which will distort the favored coordination geometry and further lead to functional loss of the protein through notable procedure, known as molecular mimicry (Dudev and Lim, 2003; Chmielowska-Bąk et al., 2013; Friedman, 2014). The Cd-binding proteins mainly have TPR, β-lactamase, and GTPase domain. Previously, it was known that Cd was involved in structural stability of these domains (Concha et al., 1997; Kajander et al., 2007). The overall functional diversity of predicted MBPs of Ott is shown in Figure 5.

GO-Enriched Network Construction

The GO-enriched biological network of the predicted MBPs consist of 179 nodes and 979 edges which were structured on 17 final kappa score groups. Figure 6 depicts the 17 groups of GO biological terms, out of which seven (oxoacid metabolic process, carboxylic acid metabolic process, cellular amino acid metabolic process, tRNA metabolic process, RNA metabolic process, RNA biosynthetic process and DNA replication, synthesis of RNA primer) were found significant. The biological node, cellular macromolecule metabolic process (GO:0044260) of groups 10 and 14, and nucleobase-containing compound metabolic process (GO:0006139) of groups 10 and 13 were the most connected GO terms with 112 and 101 links, respectively (Table S3B). There were some nodes which were associated with more than two groups (Table S3B), implying that multiple cellular processes were regulated by their associated genes.

Furthermore, the GO-enriched molecular network of predicted MBPs had 67 nodes and 101 edges with 22 kappa score groups. Among these 22, 6 groups with GO molecular terms (ribonucleotide binding, endonuclease activity, metal ion binding, purine nucleoside binding, pyrophosphatase activity and hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides) were found significant (Figure 7). The nucleotide binding (GO:0000166) node was the most linked GO term with 111 links (Table S3C). The results of GO annotation favored the domain and literature-based functional classification as large number of predicted MBPs was involved in metabolism and gene expression and regulation.

The higher number of MBPs with GO terms related to metabolism was expected. This is because by means of metabolism, microbial pathogen attains energy and nutrients which ultimately helps in their growth and survival. It is reported earlier that the MBPs involved in metabolism play important role(s) in various biosynthetic pathways, electron transport, and in signal transduction (Andreini et al., 2007a; Liu et al., 2014; Bridwell-Rabb et al., 2016). The GO network of putative MBPs was also enriched with the GO terms involved in gene expression and regulation. Previously, it was known that MBPs perform functions in DNA replication, repair, and recombination and RNA synthesis and processing (Anantharaman et al., 2002; Skaar, 2010; Porcheron et al., 2013). The functional classification and GO analysis of MBPs of Ott suggested that MBPs have diverse role(s) in its proliferation and endurance.

Toxin Prediction and Potential Therapeutic Target Prioritization

The protein toxins (virulent proteins) are produced by pathogenic bacteria in order to endure in the host environment and to improve their survival. The development of high throughput techniques helps in better understanding the role of bacterial toxins (assist in pathogenicity) at various molecular and cellular levels. We have identified that a total of 1,114 proteins (~84%) were putative virulent from the whole proteome of Ott strain Ikeda. Out of 321 putative MBPs in Ott, 245 proteins (comprised of ~22% of the total virulent proteome of Ott) were predicted as
prospective bacterial toxins that may confer to the pathogenicity. Furthermore, targeting homologous proteins may lead to cytotoxicity and cross-reactivity of drug compounds within the host (Singh et al., 2017; Sharma et al., 2018b). Therefore, pathogen-specific MBPs were identified by BLASTp search of selected 245 putative virulent MBPs against *Homo sapiens* proteins (human; taxid: 9906). We obtained 98 nonhomologous proteins which were specific to *Ott*. These 98 nonhomologous putative virulent MBPs were prioritized as potential therapeutic candidates (Table S3D). Rest 147 MBPs showed homology with the human proteins; therefore, these proteins were excluded from further analysis. It is a well-known fact that MBPs have vital role(s) in the pathogenesis of various bacterial pathogens of human (Shafeeq et al., 2013; Hameed et al., 2015). It is also documented that MBPs were a promising player in the process of drug discovery (Jamieson et al., 2003; Hameed et al., 2015; Mojica et al., 2016). Therefore, nonhomologous putative virulent MBPs of *Ott* may have the potential to act as favorable therapeutic targets.

Furthermore, to figure out most suitable drug targets being nonhomologous and being vital for virulence and survival are not only the parameters but also includes other important criteria like physiochemical properties (molecular weight, aliphatic index, theoretical pI, and GRAVY) and ability to interact with potential drugs, i.e., druggability analysis (Sharma and Kumar, 2016). The molecular weight of the proteins should be low (≤110 kDa) for acting as more significant target due to easy purification in wet lab examination (Parvege et al., 2014). We have found that molecular weight of 97 proteins range from 7.649 kDa (WP_012460740.1) to 105.999 kDa (WP_012460745.1), and a single protein (WP_012460745.1) has molecular weight 133.456 kDa. The thermostability of the proteins is positively connected with the aliphatic index, i.e., higher indices are observed in thermostable proteins than the other proteins (Ikai, 1980). We have observed that the aliphatic index of predicted virulent MBPs ranged from 55.88 (WP_012460740.1) to 128.85 (WP_012460966.1), and theoretical pI of 41 proteins was <7 and that of 57 proteins were >7. The GRAVY ranges from −1.165 for WP_012462272.1 to 0.39 for
For better interaction of proteins with water molecules, low value of GRAVY is vital. The physiochemical properties of all putative virulent nonhomologous MBPs of *Ott* are summarized in Table S3E.

The assumption behind the druggability analysis is that the druggable targets have the ability to interact with the drug or drug-like molecules (Sharma and Kumar, 2016). Therefore, to identify homologous drug targets, the shortlisted putative virulent nonhomologous 98 MBPs of *Ott* were subjected to BLASTp search at E-value of 0.00001 against the DrugBank database. Sixty proteins have satisfied the mentioned criteria and considered as significant homologues (potential druggable targets). The rest of the proteins (nonhit) were considered as novel possible drug targets which need experimental validation.

Furthermore, the functional annotation which we have done in the earlier step also supports the finding that the selected 98 putative virulent MBPs may act as probable drug targets. The shortlisted putative virulent 98 MBPs categorized into 8 broad functional classes (gene expression and regulation, metabolism, cell signaling, transport, stress response regulator, protein folding, antimicrobial resistance, and posttranslational modification).

In the class of gene expression and regulation, we have found general enzymes involved in: (i) DNA replication, repair, and recombination (DnaB-like helicase, DNA polymerases, DNA gyrase, DNA topoisomerase, DnaG, Thymidylate kinase, AP endonuclease 1, UvrABC, UvrD, uracil-DNA glycosylase, RecA, RuvC, integrase, CinA), (ii) transcription, translation, and RNA processing (RNA polymerase sigma factor, ribonucleae E/G, YbeY, NsuB, RppH, GreA/GreB, Rho, Prisomal protein N, DksA, zinc-binding ribosomal protein, IF-1, phenylalanine-tRNA ligase), and (iii) chromosome partition and condensation (ParA, ParB, IHF-like DNA-binding proteins, and tubulin/PtsZ). From the literature review, we found that these general regulatory proteins playa important roles in growth, survival, adaptation, stress responses, and virulence in most of the bacterial pathogens (Nakayama et al., 2008; Cho et al., 2010). Earlier, it is stated that architecture and conservation of bacterial DNA replication and transcription are effective target for designing broad-spectrum antibacterial agents (Robinson et al., 2012; Ma et al., 2016). Karkare et al. (2012) proposed a model for the modulation of DNA gyrase activity of *M. tuberculosis* by Ca²⁺ binding (Karkare et al., 2012). The overexpression of GreA
proteins of *E. coli* showed resistance to the toxic levels of Zn$^{2+}$ and Mn$^{2+}$ divalent metal ions, which indicate that GreA helps in their survival during harsh conditions (Stepanova et al., 2007). YbeY is a highly conserved, metal-dependent (bind to Ni$^{2+}$) protein known to play a role in the processing of 3' end of 16S rRNA, stress, and virulence regulation in bacteria (Vercruysse et al., 2014). DksA contains a zinc-finger motif and play a role in posttranscriptional regulation of quorum sensing dependent virulence genes in *P. aeruginosa* (Jude et al., 2003). TilS enzyme is involved in tRNA processing, as it catalyzes the formation of lysidine using lysine and ATP as substrate and Mg$^{2+}$ as a cofactor (Suzuki and Miyauchi, 2010). It is also known that TilS is a highly conserved and essential enzyme in bacteria and therefore act as a suitable target for broad-spectrum antimicrobial agent (Suzuki and Miyauchi, 2010). Furthermore, an inhibitor against ParA protein of *M. tuberculosis* has been designed previously, and it is stated that Mg$^{2+}$ ion significantly stimulates ATPase activity of ParA (Nisa et al., 2010). It is known that tubulin-like GTPase, FtsZ, is regulated by Ca$^{2+}$ concentration, essential for cytokinesis in bacterial cell and recognized as an excellent drug target (Erickson et al., 2010). Recently, a report indicates that a novel alpha-pyrone compound “corallopyroninA” inhibit RNA polymerase switch region of *Orientia* and therefore can act as a potent therapeutic target for scrub typhus (Kock et al., 2018).

The class of metabolism list up the proteins involved in (i) biosynthesis of bacterial cell wall [MurA, MurB, MurC, MurD, D-alanine-D-alanine ligase (DDI), DapD_Tfase_Hexep_rpt, and Aspartate kinase], (ii) amino acid metabolism [acetylglutamate kinase, glutamine synthetase (GS)], (iii) carbohydrate metabolism [glycoside hydrolase family 18 (GH), glycoside transferase family 2 (GT), N-acetylglucosamine-6-sulfate synthetase (NAGS)], (iv) nucleotide metabolism [glycoside hydrolyase family 18 (GH)], (v) fatty acid metabolism [phosphopantetheinyl transferase (PPTase)], (vi) energy generation (citrate synthase), (vii) oxidation of alcohol (PQQ-ADH). Previously, an *in silico* study stated that metabolic pathway proteins of *Ott* may act as probable target for drug discovery process (Sharma et al., 2018b). It is a well-established fact that Mur enzymes are involved in biosynthesis of bacterial cell wall using divalent metal ions as cofactor and are best intracellular therapeutic targets (Munshi et al., 2013; Moraes et al., 2015; Jukič et al., 2019). DDI enzyme uses Mg$^{2+}$ ion as cofactor and catalyzes early steps of peptidoglycan synthesis, i.e., formation of D-Ala-D-Ala and D-Ala-D-Ala dipeptides in bacteria (Tytgat et al., 2009). The enzyme DapD uses Mg$^{2+}$ ion for its stability and provides L-lysine and its immediate precursor meso-diaminopimelate, which are critical for cell-wall synthesis in most of the bacteria (Schuldt et al., 2009). It is reported previously that acetylglutamate kinase...
is a key enzyme in the synthesis of arginine, and aspartate kinase is involved in the synthesis of various amino acids (L-aspartate, threonine, methionine, isoleucine, and lysine and its precursor diaminopimelate); in addition, both the enzymes have affinity for Mg²⁺ (Ramón-Maqueos et al., 2002; Chaitanya et al., 2010). All these enzymes (Mur, DDI, DapD, aspartate kinase, and acetylglutamate kinase) are absent in human and therefore, may serve as appropriate targets for inhibition (Ramón-Maqueos et al., 2002; Schuld et al., 2009; Tytga et al., 2009; Chaitanya et al., 2010; Munshi et al., 2013; Moraes et al., 2015). Glutamine synthase catalyzes the condensation of L-glutamate and ammonia to form glutamine, requires Mg²⁺ or Mn²⁺ for reaction, plays a central role in nitrogen metabolism, and therefore can be a suitable chemotherapeutic target. Earlier, inhibition of L-glutamate and ammonia to form glutamine, requires Mg²⁺ (Wolf et al., 2014). Wolf et al. (2014) also reported the structural differences in the active site of SAICAR synthetase (PurC) of Streptococcus pneumoniae and human, which provide the basic framework for designing structure based inhibitors against bacterial PurC (Wolf et al., 2014). GH and GT enzymes are important for breakdown and synthesis of glycoside bonds, respectively. It is documented previously that these carbohydrate active enzymes are gaining importance as drug targets, and some members of these enzymes require divalent metal ions for their activity (Payne et al., 2012; Ardévol and Rovira, 2015; Schmid et al., 2016). The cell-wall hydrolase N-acetylmuramoyl-L-alanine amidase has Zn²⁺-dependent activity and is earlier known to play a role in peptidoglycan catabolic process and bacterial cell division (Vermassen et al., 2019). Cellular dUTPase have affinity for Mg²⁺ and catalyzes hydrolysis of dUTP to dUMP, therefore is necessary for DNA metabolism and bacterial survival (Hizi and Herzig, 2015). Previously, 7Fe-ferredoxin (FdxA), which contains both 3Fe–4S and 4Fe–4S cluster, is used as a drug target in M. tuberculosis (Ugalde et al., 2019). A study on P. aeruginosa stated priorly that PPTases are involved in the synthesis of fatty acid and siderophores, require Mg²⁺ ion for the reaction, and are viable target for development of antibiotics (Finking et al., 2002).

The cell signaling class was found to be enriched in HD domain. The other domains found in this class were OmpR/PhoB-type DNA binding, GGDFE, EAL, and phospholipase D/transphosphatidylase (Finking et al., 2002). The role of HD domain has already discussed in the above section. OmpR/PhoB DNA-binding proteins act as two-component signal transduction transcriptional regulator and have the ability to bind Mg²⁺ ion, which act as cofactor for its catalytic activity (Sola et al., 2006). Previously, it is documented that the two-component systems serve as satisfactory target for drug designing because of their inference in virulence, resistance to the drug, their universality in bacteria, and their absence in humans (Blanco et al., 2002). It is a well-known fact that cyclic-GMP signaling in bacteria regulates wide range of functions like adhesion, biofilm formation, and virulence (Ryan et al., 2006). Both GGDFE and EAL protein domains are involved in cyclic Di-GMP signaling; GGDFE domain synthesizes cyclic di-GMP; on the other side, EAL domain catalyzes cyclic-GMP hydrolysis; and both domains require divalent metal ion as cofactor (Mg²⁺ or Mn²⁺) (Ryan et al., 2006). Earlier, it is reported that the bacterial phospholipase D is involved in the hydrolysis of phospholipids and generates metabolites that act as secondary messengers, which further play roles in host cell invasion, modulation of lipid content of host cell membrane, and pathogenesis (Flores-Díaz et al., 2016). The Zn²⁺-binding ability of phospholipase D has also been reported earlier in Bacillus subtilis (Garutskas et al., 1977).

The periplasmic bacterial soluble-binding protein (SBP), type-IV secretion protein TraC, MgtE intracellular domain, Tol-Pal system/ TolQ, and cation efflux protein were mentioned under transport category. A study on periplasmic SBP of Paracoccus denitrificans indicates that SBP binds to Zn ion, helps in its transport through ABC transporter, and therefore, can act as potent antibacterial target (Neupane et al., 2017). Earlier, it is known that Otr have massively proliferating conjugaive transfer system (Cho et al., 2007), and TraC protein was probably involved in conjugal transfer, synthesis, and assembly of F conjugaive pilus (Schandel et al., 1992). It is reported previously in P. aeruginosa that inner membrane MgtE protein helps in the transport of magnesium ion, stimulates rsmYZ transcription to inhibit gene expression of type-III secretion system, and therefore, act as virulence modulator (Chakravarty et al., 2017). Earlier, it is stated that the Tol-Pal system acts as ion potential-driven molecular motor in a wide range of Gram-negative bacteria, which help them to maintain the stability of their outer membrane and export of cell envelope component (Cascales et al., 2001). The cation efflux protein was reported earlier to raise the tolerance against Zn, Cd, Co, and Ni and help to remove the excess of these metal ions in order to maintain metal homeostasis (Stähler et al., 2006).

We have found Ppx/GppA phosphatase, thioredoxin reductase, and divalent ion tolerance protein (CutA) in the category of stress response regulator. Earlier, a study on Ppx/GppA phosphatase of Aquifex aeolicus indicated that these enzymes require metal ions (Ca²⁺) for their catalysis and play crucial roles in bacterial stringent response triggered by starvation (Kristensen et al., 2004). The roles of thioredoxin reductase in numerous cellular processes and regulation of oxidative stress are well known. Furthermore, earlier studies also reported that thioredoxin reductases are viable target for inhibition using antibiotics and metal binding (Rolling-Genet et al., 2004; Lu et al., 2013). The small CutA protein is universally distributed in a broad range of bacteria, and its role in divalent metal tolerance (uptake, storage, delivery, and efflux) has been documented earlier in E. coli (Rensing and Grass, 2003). We found two metallo-β-lactamase in our study that putatively have binding site for Zn, Mn, and Ca and probably involved in antimicrobial resistance. It is documented formerly that metallo-β-lactamase has affinity for cations (Zn, Fe, Mn) which acts as cofactor for the enzyme (Palzzkill, 2013). Earlier, a Ca-EDTA inhibitor was found efficient for metallo-β-lactamase in a mouse model of P. aeruginosa pneumonia (Aoki et al., 2010).

Protein folding category contains a copper chaperones PCu(A) having affinity for Cu²⁺ and a chaperoin 10 protein having affinity for Ca²⁺. The role of copper chaperones PCu(A) is earlier noticed in the biogenesis and assembly of respiratory enzyme complexes (Thompson et al., 2012). Roberts et al. (2003) reported that chaperonin 10 plays a role in folding of structural protein domains, transport of proteins, ATP, and peptide (Roberts et al., 2003). Furthermore, it is also stated that chaperonin 10 acts as a virulent
factor and helps to maintain stress response in *M. tuberculosis* (Roberts et al., 2003). In the class of posttranslational modification, we have found tRNA threonylcarbamoyl adenosine modification protein (TsaE). It is reported previously that TsaE interferes in pre- or postcatalytic step of *t*RNA modification and has affinity for Mg$^{2+}$, and its mechanism of action and binding mode make it a suitable antimicrobial target (Missoury et al., 2018). These findings and facts supported our study that the predicted virulent nonhomologous MBPs may act as appropriate targets for developing drug against the pathogen.

**CONCLUSION**

In the presented work, we have integrated multiple bioinformatics tools aimed to identify putative MBPs of zoonotic pathogen *Ott*. The analysis showed that about a quarter of pathogen proteome was enriched with metals and was pervasive with magnesium. The prediction of MBPs in different subcellular compartments of the bacterial cell has documented that most of the MBPs localized in the cytoplasm. The involvement of MBPs in diverse cellular and biological processes has been noted through their functional domain classification. Furthermore, the druggability analysis of putative MBPs indicate that these may act as druggable targets. In conclusion, this study provides the repository of putative MBPs which might serve as primer for experimental validation. These putative MBPs may have the ability to act as therapeutic targets for developing metal-based antimicrobial agent against the pathogenic *Ott*.

**REFERENCES**

Akcapinar, G. B., and Sezeroman, O. U. (2017). Computational approaches for de novo design and redesign of metal-binding sites on proteins. *Biosci. Rep.* 37, BSR20160179. doi: 10.1042/BSR20160179

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi: 10.1016/0022-2836(90)80360-2

Anantharaman, V., Koonin, E. V., and Aravind, L. (1998). The HD domain defines a new superfamily of metal-dependent phosphohydrolases. *Nucleic Acids Res.* 30, 1427–1464. doi: 10.1093/nar/30.7.1427

Andréini, C., Banci, L., Bertini, I., Elmi, S., and Rosato, A. (2007b). Occurrence of copper proteins through the three domains of life: a bioinformatic approach. *J. Proteome Res.* 7, 209–216. doi: 10.1021/pr070480u

Andréini, C., Banci, L., Bertini, I., and Rosato, A. (2007a). Non-heme iron through the three domains of life. *Proteins* 67, 317–324. doi: 10.1002/prot.21324

Andreini, C., Banci, L., Bertini, I., Elmi, S., and Rosato, A. (2007b). Occurrence of copper proteins through the three domains of life: a bioinformatic approach. *J. Proteome Res.* 7, 209–216. doi: 10.1021/pr070480u

Aravind, L., and Koonin, E. V. (1998). The HD domain defines a new superfamily of metal-dependent phosphohydrolases. *Trends Biochem. Sci.* 23, 469–472. doi: 10.1016/S0968-0004(98)01293-6

Bridwell-Rabb, J., Kang, G., Zhong, A., Liu, H., and Drennan, C. L. (2016). An HD domain phosphohydrolase active site tailored for oxetanocin-A biosynthesis. *Proc. Natl. Acad. Sci.* 113, 13750–13755. doi: 10.1073/pnas.1613610113

Blanco, A. G., Sola, M., Gomis-Rüth, F. X., and Coll, M. (2002). Tandem DNA motifs. *J. Bacteriol.* 184, 136–144. doi: 10.1128/JB.00276-15

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., et al. (2009). ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–1093. doi: 10.1093/bioinformatics/btp101

Breyer, A. R., VieBrock, L., Rodino, K. G., Miller, D. P., Tegels, B. K., Marconi, R. T., et al. (2015). *Orientia tsutsugamushi* strain Ikeda ankyrin repeat-containing proteins recruit SCF ubiquitin ligase machinery via povixir-like F-box motifs. *J. Bacteriol.* 197, 3097–3109. doi: 10.1128/JB.00276-15

Bridwell-Rabb, J., Kang, G., Zhong, A., Liu, H., and Drennan, C. L. (2016). An HD domain phosphohydrolase active site tailored for oxetanocin-A biosynthesis. *Proc. Natl. Acad. Sci.* 113, 13750–13755. doi: 10.1073/pnas.1613610113

Campanacci, V., Mukherjee, S., Roy, C. R., and Cherfils, J. (2013). Structure of the Legionella effector AnkX reveals the mechanism of phosphocholine transfer by the Fic domain. *EMBO J.* 32, 1469–1477. doi: 10.1038/emboj.2013.82

**DATA AVAILABILITY**

Publicly available datasets were analyzed in this study. This data can be found here: [https://www.ncbi.nlm.nih.gov/refseq/](https://www.ncbi.nlm.nih.gov/refseq/)

**AUTHOR CONTRIBUTIONS**

DS and SKV conceived the idea and designed the experiments. DS predicted the MBPs of *Ott*. AS performed functional classification and clustering. DS predicted the proteins 3D structure. AS and DS performed mining of MBPs of *Ott* and analyzed the data. DS wrote the manuscript. SKV and BS checked the manuscript. All authors read and approved the manuscript.

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**SUPPLEMENTARY MATERIAL**

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minimal medium. Appl. Environ. Microbiol. 75, 2720–2726. doi: 10.1128/AEM.02738-08

Klein, J. F., and Levinson, O. (2011). Bacterial ATP-driven transporters of transition metals: physiological roles, mechanisms of action, and roles in bacterial virulence. Metallomics 3, 1098–1108. doi: 10.1039/c1mt00075j

Ko, Y., Cho, N.-H., Cho, B.-A., Kim, I.-S., and Choi, M.-S. (2011). Involvement of Ca2+ signaling in intracellular invasion of non-phagocytic host cells by Orientia tsutsugamushi. Microb. Pathog. 50, 326–330. doi: 10.1016/j.micpath.2011.02.007

Kock, F., Hauptmann, M., Osterloh, A., Schäberle, T. F., Poppert, S., Frickmann, H., et al. (2018). Orientia tsutsugamushi is highly susceptible to the RNA polymerase switch region inhibitor corallopyronin A in vitro and in vivo. Antimicrob. Agents Chemother. 62, e01732–e01717. doi: 10.1128/AAC.01732-17

Kristensen, O., Laurberg, M., Liljas, A., Kastrup, J. S., and Gajhede, M. (2004). Structural characterization of the stringent response related exopyrophosphatase/guanosine pentaphosphate phosphohydrolase protein family. Biochemistry 43, 8894–8900. doi: 10.1021/bi040983c

Lin, Y. F., Cheng, C. W., Shih, C. S., Hwang, J. K., Yu, C. S., and Lu, C. H. (2016). MIB: metal ion binding-site prediction and docking server. J. Chem. Inf. Model. 56, 2287–2291. doi: 10.1021/acs.jcim.6b00407

Liu, J., Chakraborty, S., Hossein zadde, P., Yu, Y., Tian, S., Petrik, I., et al. (2014). Metalloproteins containing cytochrome, iron-sulfur, or copper redox centers. Chem. Rev. 114, 4366–4469. doi: 10.1021/cr400479b

Lu, C.-H., Lin, Y.-F., Lin, J.-J., and Yu, C.-S. (2012). Prediction of metal ion-binding sites: the fragment-based information method. PLoS One 7, e39252. doi: 10.1371/journal.pone.0039252

Lu, J., Vlamin-Gardikas, A., Kandasamy, K., Zhao, R., Gustafsson, T. N., Engstrand, L., et al. (2013). Inhibition of bacterial thiolredoxin reductase: an antibiotic mechanism targeting bacteria lacking glutathione. FASEB J. 27, 1394–1403. doi: 10.1096/fj.12-223305

Ma, C., Yang, X., and Lewis, P. J. (2016). Bacterial transcription as a target for antibacterial drug development. Microbiol. Mol. Biol. Rev. 80, 139–160. doi: 10.1128/MMBR.00055-15

Ma, L., Terwilliger, A., Marcess, A. W., Valle, B., Falchuk, H., Cair, G., et al. (2015). Iron and zinc exploitation during bacterial pathogenesis. Metallomics 7, 1541–1554. doi: 10.1039/C5MT00170F

Ma, Z., Jacobsen, F. E., and Giedroc, D. P. (2016). Metal coordination chemistry of bacterial metal transport and sensing. Chem. Rev. 116, 4644–4681. doi: 10.1021/acs.chemrev.6b00279

Mahajan, S. K., Rolain, J. M., Kanga, A., and Raoult, D. (2010). Scrub typhus. Annu. Rev. Genet. 44, 67–91. doi: 10.1146/annurev-genet-120215-035146

Malczewski, G., Zalewski, A., Sadowski, M., Tornarolski, A., and Dozois, C. M. (2013). Copper, zinc, and manganese transport and regulation in pathogenic Enterobacteria: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front. Cell. Infect. Microbiol. 3, 90. doi: 10.3389/fcimb.2013.00090

Maret, W. (2016). The metals in the biological periodic system of the elements: physiological roles, mechanisms of action, and roles in disease. Metalloproteome of Orientia tsutsugamushi. Ikeda. Frontiers in Genetics | www.frontiersin.org 15 September 2019 | Volume 10 | Article 797

Roberts, M. M., Gare, A. R., Fossati, G., Mascagni, P., Coates, A. R. M., and Brinkman, F. S. L. (2005). Assessing the precision of high-throughput computational and laboratory approaches for the genome-wide identification of protein subcellular localization in bacteria. BMC Genomics 6, 162. doi: 10.1186/1471-2164-6-162

Rey, S., Gardy, J. L., and Brinkman, F. S. L. (2005). Assessing the precision of high-throughput computational and laboratory approaches for the genome-wide identification of protein subcellular localization in bacteria. BMC Genomics 6, 162. doi: 10.1186/1471-2164-6-162

Rey, S., Gardy, J. L., and Brinkman, F. S. L. (2005). Assessing the precision of high-throughput computational and laboratory approaches for the genome-wide identification of protein subcellular localization in bacteria. BMC Genomics 6, 162. doi: 10.1186/1471-2164-6-162

Palzkill, T. (2013). Metallo-β-lactamase structure and function. J. Biol. Chem. 288, 36322–36330. doi: 10.1074/jbc.M112.402149

Porcheron, G., Garénaux, A., Proulx, J., Sabri, M., and Dozois, C. M. (2013). Iron, copper, zinc and manganese transport and regulation in pathogenic Enterobacteria: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front. Cell. Infect. Microbiol. 3, 90. doi: 10.3389/fcimb.2013.00090

Pruitt, K. D., Tatusova, T., and Maglott, D. R. (2006). NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res. 35, D61–D66. doi: 10.1093/nar/gkq242

Rensing, C., and Grass, G. (2003). Escherichia coli mechanisms of copper homeostasis in a changing environment. FEMS Microbiol. Rev. 27, 197–213. doi: 10.1016/S0168-6445(03)00049-4

Remy, L., Carrière, M., Derré-Bobillot, A., Martini, C., Sanguineti, M., and Boreé-Durant, E. (2013). The Sphingobacterium aureus OppA ABC transporter imports nickel and cobalt in zinc-depleted conditions and contributes to virulence. Mol. Microbiol. 87, 730–743. doi: 10.1111/mmi.12126

Remy, L., Carrière, M., Derré-Bobillot, A., Martini, C., Sanguineti, M., and Boreé-Durant, E. (2013). The Sphingobacterium aureus OppA ABC transporter imports nickel and cobalt in zinc-depleted conditions and contributes to virulence. Mol. Microbiol. 87, 730–743. doi: 10.1111/mmi.12126

Renesto, P., Ogata, H., Audic, S., Claverie, J.-M., and Raoult, D. (2005). Some lessons from Rickettsia genomics. FEMS Microbiol. Rev. 29, 99–117. doi: 10.1016/j.femsre.2004.09.002

Rensing, C., and Grass, G. (2003). Escherichia coli mechanisms of copper homeostasis in a changing environment. FEMS Microbiol. Rev. 27, 197–213. doi: 10.1016/S0168-6445(03)00049-4

Roberts, M. M., Coker, A. R., Fossati, G., Mascagni, P., Coates, A. R. M., and Wood, S. P. (2003). Mycobacterium tuberculosis chaperonin 10 heat shock proteins...
responsive to antibiotics in northern Thailand. *Lancet* 348, 86–89. doi: 10.1016/S0140-6736(96)02501-9

Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., et al. (2017). DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* 46, D1074–D1082. doi: 10.1093/nar/gkx1037

Wolf, N. M., Abad-Zapatero, C., Johnson, M. E., and Fung, L.-M. (2014). Structures of SAICAR synthetase (PurC) from *Streptococcus pneumoniae* with ADP, Mg2+, AIR and Asp. *Acta Crystallogr. D Biol. Crystallogr.* 70, 841–850. doi: 10.1107/S139900471303366X

Wommer, S., Rival, S., Heinz, U., Galleni, M., Frère, J.-M., Franceschini, N., et al. (2002). Substrate-activated zinc binding of metallo-β-lactamases physiological importance of the mononuclear enzymes. *J. Biol. Chem.* 277, 24142–24147. doi: 10.1074/jbc.M202467200

Xu, G., Walker, D. H., Jupiter, D., Melby, P. C., and Arcari, C. M. (2017). A review of the global epidemiology of scrub typhus. *PLoS Negl. Trop. Dis.* 11, e0006062. doi: 10.1371/journal.pntd.0006062

Yu, C.-S., Lin, C.-J., and Hwang, J.-K. (2004). Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions. *Protein Sci.* 13, 1402–1406. doi: 10.1110/ps.03479604

Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., et al. (2010). PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* 26, 1608–1615. doi: 10.1093/bioinformatics/btq249

Zhu, P.-P., Li, W.-C., Zhong, Z.-J., Deng, E.-Z., Ding, H., Chen, W., et al. (2015). Predicting the subcellular localization of mycobacterial proteins by incorporating the optimal tripptides into the general form of pseudo amino acid composition. *Mol. Biosyst.* 11, 558–563. doi: 10.1039/C4MB00645C

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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