

Interpreting the function of VapBC Toxin–Antitoxin Family of *Mycobacterium tuberculosis* through *in Silico* Approaches in an *E. coli* System

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Abstract

Mycobacterium tuberculosis (Mtb) harbors a great number of Toxin-Antitoxin (TA) systems, in which half of them belong to virulence linked proteins B and C (VapBC) family that has a typical PilT N-terminus domain and ribonuclease activity. Functional perceptions into Mtb VapBC TA modules untangled their function in adaptation to numerous host-mediated stressors, including oxidative/nitrosative, chemical and nutrient starvation concomitant with multidrug tolerance and establishment of persistence. To recognize the complexities of Mtb's pathogenesis, absolute cellular targets of 19 VapC(s) were monitored. Some display a shared ribonuclease activity, while others harbor tRNase and 23S rRNA cleavage activity. The comprehensive functional description of VapBC4, VapBC12 and VapBC22, comprising *in vivo* deletion mutant studies exposed their part in Mtb's virulence/persistence. The VapC22 mutant was lessened for Mtb's growth in mice and provoked a decreased T_H1 response, while mice infected with VapC12 mutant exhibited a considerably higher bacillary load and pro-inflammatory response than the wild type, displaying a hyper-virulent phenotype. Supplementary experimental studies are required to interpret the functional role of VapBC systems and disentangle their cellular goals. Taken together, Mtb VapBC TA systems appear to be encouraging drug targets owing to their main part in persisting stressors, antibiotic resistance and resolution.

Keywords: *Mycobacterium tuberculosis*; toxin-antitoxin system; VapBC family; VapC toxin.

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I. Introduction

Tuberculosis (TB), caused by the pathogenic bacterium *Mycobacterium tuberculosis*, remains one of the deadliest infectious diseases worldwide. Despite decades of research and control efforts, TB continues to claim more than 1.5 million lives annually, making it a major global public health concern (Reid et al., 2019; Miggiano et al., 2020). The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has further complicated treatment strategies and highlighted the urgent need for novel therapeutic targets (Dwivedi et al., 2008; Dwivedi et al., 2011; Allué-Guardia et al., 2021).

One promising area of study lies in bacterial toxin–antitoxin (TA) systems (Pizzolato-Cezar et al., 2023). Originally identified on plasmids as plasmid maintenance or “addiction” modules, TA systems were later found to be widely distributed across bacterial chromosomes (Sonika et al., 2023). Typically organized in operons, these systems consist of a stable toxin and a labile antitoxin. The antitoxin neutralizes the toxin under normal conditions, while the toxin can inhibit essential cellular processes—leading to growth arrest or cell death—when the antitoxin is degraded (Sonika et al., 2023). Chromosomal TA systems have been implicated in diverse biological functions, including stress adaptation, biofilm formation, phage defence, persistence, and virulence (Rosendahl et al., 2020). Their widespread presence in pathogenic bacteria suggests an important role in infection establishment, intracellular survival, host adaptation, and chronic disease progression (Kamruzzaman et al., 2021).

Remarkably, *M. tuberculosis* possesses a very high number of toxin-antitoxin (TA) systems in its chromosome, 79 in total, regrouping both well-known (68) and novel (11) families, with some of them being strongly induced in drug-tolerant persisters (Sala et al., 2014). These operons are dispersed throughout the genome and are thought to contribute to pathogenesis and persistence within the host. Interestingly, all TA systems in *M. tuberculosis* belong exclusively to the type II class, where both toxin and antitoxin are proteins that directly interact (Khan et al., 2023).

Toxin–antitoxin (TA) systems are classified into eight types (I–VIII) based on the mechanism by which the antitoxin neutralizes the toxin (Sonika et al., 2023). In type I systems, an RNA antitoxin binds to the toxin

mRNA, preventing its translation. In type II systems, a protein antitoxin directly interacts with the toxin protein, forming a complex that inhibits its activity. In type III systems, the RNA antitoxin associates with the toxin protein and blocks its function. In type IV systems, the protein antitoxin binds to the toxin's cellular target rather than the toxin itself, thereby providing indirect inhibition. In type V systems, the antitoxin protein degrades toxin mRNA, preventing toxin synthesis. In type VI systems, the antitoxin protein recruits proteases by serving as a proteolytic adaptor, leading to toxin degradation. In type VII systems, the antitoxin protein inhibits toxin activity through post-translational modification of the toxin. Finally, in type VIII systems, the antitoxin RNA interferes with toxin expression either by repressing its transcription or by directly binding toxin RNA to promote its degradation. (Qiu et al., 2022)

Within type II systems, the VapBC family (virulence-associated proteins) represents the largest group. *M. tuberculosis* encodes 51 VapBC pairs, making it the most expanded TA family in its genome (Thakur et al., 2024). VapC toxins contain a conserved PIN domain associated with RNase activity, while VapB proteins function as their cognate antitoxins. (Kang, 2023) Despite their abundance, the physiological roles, evolutionary relationships, and functional diversity of VapBC systems remain poorly understood. Given their potential contribution to persistence, stress response, and drug tolerance, VapBC TA modules have emerged as attractive candidates for *in silico* and experimental characterization (Singh et al., 2021).

The present study focuses on the *in-silico* characterization of the VapBC TA family of *M. tuberculosis* using the *Escherichia coli* system as a model framework. Since *E. coli* is a well-established host for functional studies of TA modules, computational analysis and comparative modelling can provide valuable insights into the structural, evolutionary, and functional aspects of VapBC proteins. Therefore, the present study was undertaken with an approach that not only helps decipher the role of VapBC systems in Mtb pathogenesis but also identifies potential therapeutic targets for combating drug-resistant TB.

II. Materials and Methods

2.1. Retrieval of VapBC Sequences

Representative VapBC toxin–antitoxin (TA) pairs from *Mycobacterium tuberculosis* H37Rv data was retrieved from Toxin-antitoxin database (TADB) (Shao et al., 2010). Five pairs were retrieved using their respective Rv numbers. Both nucleotide and protein sequences were downloaded in FASTA format from the NCBI database for downstream analysis. To ensure accuracy, antitoxin (VapB) and toxin (VapC) genes were cross-verified for their genomic organization, with the antitoxin typically positioned upstream of the toxin.

2.2. Multiple Sequence Alignment and Domain Analysis

Protein sequences of VapB and VapC families were aligned separately to assess sequence conservation. Multiple sequence alignments were performed using Clustal Omega (EMBL-EBI) (Kang et al., 2017). Functional domains and conserved regions were identified through InterProScan database (Blum et al., 2024). Particular emphasis was placed on detecting the PIN-domain ribonuclease motifs in VapC and DNA-binding motifs (RHH/HTH) in VapB.

2.3. Phylogenetic Analysis

Phylogenetic relationships among VapB and VapC proteins were investigated using MEGA12 (Kumar et al., 2024). Protein sequences were first aligned using the MUSCLE algorithm (Edgar, 2004), and evolutionary trees were constructed using the Neighbor-Joining method with Poisson correction. Reliability of clustering was tested by bootstrap analysis with 1000 replications. The resulting phylogenetic trees were exported for graphical presentation and interpretation of evolutionary divergence (Fernández et al., 2023).

2.4. Structural Modeling of VapBC Proteins

Three-dimensional structures of VapB and VapC proteins were obtained either from the PDB Database. Structural validation was performed using SAVES server, where Ramachandran plots and stereochemical quality parameters were evaluated. (Singh et al., 2025). Final structures were visualized in PyMOL, with attention given to active sites of VapC and DNA-binding motifs of VapB.

2.5. Protein–Protein Docking of VapB and VapC

To investigate molecular interactions between VapB antitoxin and VapC toxin, docking simulations were carried out using ClusPro (Kozakov et al., 2017). Antitoxins were designated as receptors and toxins as ligands. The top-ranked docked complexes were analyzed for binding energy, hydrogen bonds, and interface residues. Structural interactions were visualized in Discovery studios visualizer to highlight key contact regions responsible for neutralization of toxin activity.

2.6. Codon Usage Analysis and Expression Feasibility

Codon optimization was performed to assess expression potential of VapB and VapC genes in heterologous hosts (*E. coli* K12). DNA sequences were analyzed using JCat to calculate Codon Adaptation Index (CAI) and optimized for target hosts (Grote et al., 2005).

III. Results and discussion

3.1. Identification and Retrieval of VapBC Sequences

A total of five VapBC operon pairs were selected from *Mycobacterium tuberculosis* H37Rv, based on prior literature and database annotations (Table 1). The protein sequences for VapB (antitoxins) and VapC (toxins) were retrieved using TADB (Toxin – Antitoxin Database) and cross-referenced with NCBI Protein to confirm annotations. The selected pairs included: vapB1–vapC1 vapB2–vapC2, vapB3–vapC3, vapB26–vapC26, and vapB28–vapC28.

Table 1. Summary of selected VapBC pairs and their gene identifiers.

Vap B Gene (Antitoxin)	Vap C Gene (Toxin)	Rv Numbers	Protein Length (aa)
Vap B1	Vap C	Rv0064A-Rv0065	80-136
Vap B2	Vap C	Rv0300-Rv0301	74-144
Vap B3	Vap C	Rv0550c-Rv0549c	89-140
Vap B26	Vap C	Rv0581-Rv0582	72-138
Vap B28	Vap C	Rv0608-Rv0609	82-136

The sequences were saved in FASTA format and organized into VapB and VapC categories for downstream analyses. In prokaryotes, cognate toxin-antitoxin pairs have long been known, but no three-dimensional structure has been available for any given complex from *Mycobacterium tuberculosis* (Dwivedi et al., 2008; Millau et al., 2009;). Here we report the crystal structure and activity of a member of the VapBC family of complexes from *M. tuberculosis*. The toxin VapC-5 is a compact, 150 residues, two domain α/β protein. Bent around the toxin is the VapB-5 antitoxin, a 33-residue α -helix. Assays suggest that the toxin is an Mg-enabled endoribonuclease, inhibited by the antitoxin. The lack of DNase activity is consistent with earlier suggestions that the complex represses its own operon (Kang et al., 2020). Furthermore, analysis of the interactions in the binding of the antitoxin to the toxin suggest that exquisite control is required to protect the bacteria cell from toxic VapC-5.

3.2. Multiple Sequence Alignment of VapB and VapC Proteins

Protein sequences of selected VapB and VapC proteins were aligned separately using Clustal Omega to identify conserved regions and functionally important residues within each family.

In the VapC alignment, Aspartic acid (D) and Alanine (A) residues were found to be fully conserved across all analysed sequences, as indicated by the (*) symbol in the Clustal Omega output. Several other residues—Valine (V), Isoleucine (I), Methionine (M), Phenylalanine (F), Aspartic acid (D), and Glycine (G)—displayed strong (:) or weak (.) similarity, reflecting partial conservation among VapC homologs (Figure 1). The conservation of Aspartic acid (D), an acidic residue, is particularly significant, as acidic amino acids often contribute to metal ion coordination and catalytic activity within the PIN domain. This is a correct interpretation of common findings in studies of the VapC toxin family. The highly conserved aspartate (D) residue in VapC proteins is indeed widely recognized as critical for their ribonuclease (RNase) function (Sharrock et al., 2018). It typically acts as a key catalytic residue within the active site, facilitating the cleavage of specific RNA substrates to induce bacterial stasis or cell death. This suggests that the conserved D residue plays a crucial role in the RNase function of VapC proteins.

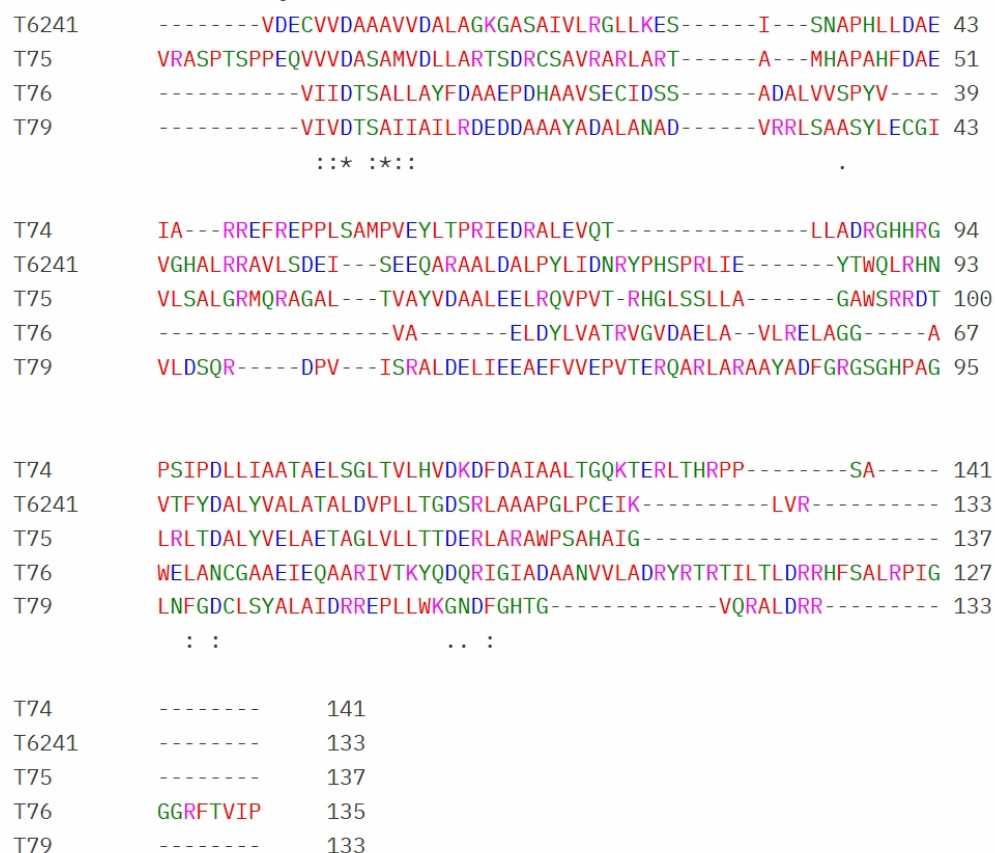


Figure 1: Multiple sequence alignment of VapC proteins highlighting conserved residues. Fully conserved residues are indicated by (*), strongly similar residues by (:), and weakly similar residues by (.)

In the VapB alignment, only Glycine (G) was identified as a fully conserved residue, while Methionine (M), Valine (V), Leucine (L), Serine (S), Aspartic acid (D), and Glutamic acid (E) showed strong or weak similarity among the sequences (Figure 2). The presence of these partially conserved residues may contribute to maintaining the overall structure of the Ribbon-Helix-Helix (RHH) domain, which is characteristic of VapB proteins and important for DNA binding and interaction with the VapC toxin (Jin et al., 2015).

CLUSTAL O(1.2.4) multiple sequence alignment

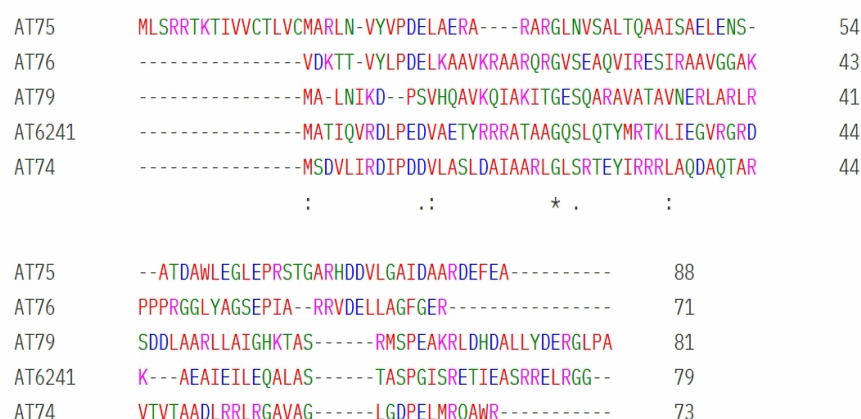


Figure 2. Multiple sequence alignment of VapB proteins highlighting conserved residues. Fully conserved residues are indicated by (*), strongly similar residues by (:), and weakly similar residues by (.)

Overall, the VapC alignment displayed conservation of residues likely involved in catalytic activity, whereas VapB showed conservation patterns consistent with structural stability and regulatory function—highlighting the complementary roles of these two components in the VapBC toxin–antitoxin system. Notwithstanding the ubiquity of VapBC systems and their critical role in the regulation of gene expression, few functional studies have addressed the details of VapB–VapC interactions (Aakre et al., 2013; Jin et al., 2015). Here authors report on the results of experiments designed to identify molecular determinants of the specificity of the *Mycobacterium tuberculosis* VapB4 antitoxin for its cognate VapC4 toxin. The results identify the minimal domain of VapB4 required for this interaction as well as the amino acid side chains required for binding to VapC4. These findings have imperative implications for the evolution of VapBC toxin–antitoxin systems and their prospective as targets of small-molecule protein–protein interaction inhibitors.

3.3. Domain and Motif Analysis

Functional domain and motif analysis was conducted using InterProScan and cross-validated with Pfam and SMART databases to characterize the structural and functional elements within VapB and VapC proteins (Table 2). All VapC proteins analyzed were found to contain a highly conserved PIN (PiIT N-terminus) domain, typically spanning residues ~50–120, depending on sequence length. The highly conserved PIN domain in VapC proteins functions primarily as an endoribonuclease (RNase) (Matelska et al., 2017). Up to date, considerable effort has been made to characterize structures and catalytic mechanisms of the PIN-like nucleases. However, our knowledge about structure and functions of PIN-like domains is largely biased towards VapC and FEN-like domains, which—being considerably distinct—do not provide insights into the roles of individual structural elements and subtle local sequence differences in the substrate specificity. It would be of great interest to study in detail how the structural insertions influence substrate recognition and catalytic mechanism of the nucleases, in particular in the least studied groups, i.e., NYN, PRORP, and Mut7-C. This nuclease activity is crucial because VapC proteins are typically the toxic components of bacterial toxin–antitoxin (TA) systems, and their function is to cleave RNA and arrest bacterial growth under stress conditions. The PIN domain is a well-characterized ribonuclease catalytic domain, commonly present in prokaryotic toxins.

In contrast, VapB proteins exhibited features consistent with DNA-binding and antitoxin functions. Most VapB homologs contained either a Ribbon-Helix-Helix (RHH) or a Helix-Turn-Helix (HTH) motif in the N-terminal region. These motifs are typical of small, dimeric DNA-binding proteins that recognize palindromic operator sequences to repress transcription of toxin–antitoxin operons. The C-terminal region of VapB proteins is more variable and often predicted to form intrinsically disordered segments, which facilitate flexible binding to VapC toxins for neutralization.

Table 2. Domain annotations for VapB and VapC proteins.

Protein	Domain	Position	Source
VapB1	RHH(IPR010985) FitA-like-RHH(IPR053853)	2-67 2-37	InterProScan InterProScan
VapC1	PIN(IPR044153) PIN(IPR029060) PIN(IPR002716)	37-125 4-126 5-124	InterProScan InterProScan InterProScan
VapB2	RHH(IPR010985) RHH Protein, CopG family (IPR002145) Pfam (PF01402)	3-54 9-38	InterProScan InterProScan Pfam
VapC2	PIN(IPR029060) PIN(IPR002716)/ Pfam (PF01850) PIN-mt VapC3-VapC21liKE CDD cd18755	6-123 7-125 7-135	InterProScan InterProScan/Pfam InterProScan
VapB3	Pfam (PF07362)/Post segregation antitoxin CcdA (IPR009956)	19-61	Pfam
VapC3	PIN(IPR029060) PIN(IPR002716) PIN-Pae0151-like (IPR044153)	12-131 12-129 13-129	InterProScan InterProScan InterProScan
VapB26	RHH(IPR010985) Pfam (PF01402), RHH Protein, Cop family (IPR002145)	1-58 4-37	Pfam Pfam
VapC26	PIN(IPR029060) PIN(IPR002716) PIN-mtVapC26-like CDD cd18696	1-123 1-121 2-133	InterProScan InterProScan InterProScan

VapB28	Pfam (PF07704)/(IPR011660)	1-80	Pfam
VapC28	PIN(IPR029060) PIN(IPR002716)	1-128 1-124	InterProScan InterProScan

Taken together, these results confirm that VapB and VapC proteins retain their canonical toxin–antitoxin domain architectures. The conservation of key motifs, particularly the PIN catalytic residues and RHH DNA-binding region, implies strong functional and evolutionary constraints maintaining their toxin–antitoxin pairing across species (Bahl et al., 2025).

3.4. Phylogenetic Relationships

To explore the evolutionary relationships and diversification patterns of VapB and VapC proteins, Neighbor-Joining (NJ) phylogenetic trees were constructed in MEGA12 using MUSCLE-aligned sequences. The Poisson correction model was applied to estimate amino acid substitutions, and bootstrap analysis with 1000 replicates was performed to evaluate the reliability of each branch (Kumar et al., 2024).

The VapC phylogenetic tree revealed the presence of multiple distinct clades, reflecting both species-specific and cross-species clustering. Closely related VapC proteins grouped together with high bootstrap support (>80%), indicating recent divergence or conservation within bacterial lineages. Interestingly, some VapC sequences formed distinct sub-clusters separate from the main toxin clades, suggesting possible functional specialization or horizontal gene transfer events.

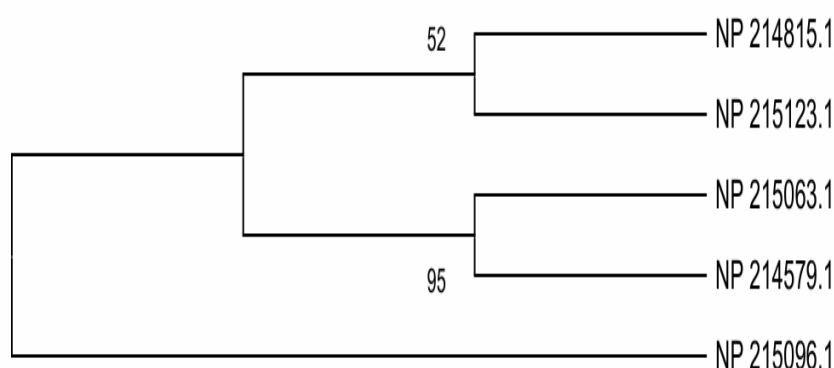


Figure 3. Phylogenetic tree of Vap C Proteins.

Comparative inspection of the VapC alignment indicated that these divergent branches often carried substitutions in conserved acidic residues or insertions/deletions in the PIN domain loops, which may influence substrate specificity or catalytic efficiency (Figure 3). This suggests that VapC proteins, while structurally conserved, may have evolved to target different RNA substrates or respond to distinct stress conditions.

The VapB tree displayed a similar clustering pattern, with sequences forming species-specific subfamilies that largely mirrored the topology of the corresponding VapC tree (Figure 4). This co-clustering supports the notion of co-evolution between VapB and VapC pairs, a common feature of toxin-antitoxin systems. VapB variants that appeared phylogenetically divergent often contained extended C-terminal regions or modified DNA-binding motifs, possibly reflecting adaptation to regulate diverse promoter sequences or interact with structurally variable VapC partners (Matelska et al., 2017).

3.5. Protein Structure prediction of VapBC Proteins

The three-dimensional (3D) structures of selected VapB (antitoxin) and VapC (toxin) proteins from *Mycobacterium tuberculosis* were modeled using the SWISS-MODEL server. Among the five VapB–VapC sequence pairs analyzed, experimentally resolved structures were available in the Protein Data Bank (PDB) for only two pairs, namely VapB2–VapC2 (PDB ID: 3H87) and VapB26–VapC26 (PDB ID: 5X3T) (Millau et al., 2009; Damiano et al., 2024). Therefore, these two structures were selected for subsequent structural validation and quality assessment using the SAVES server.

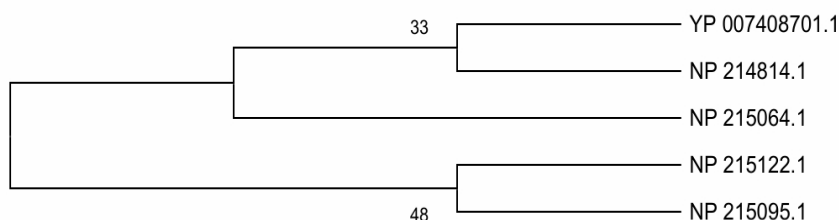


Figure 4. Phylogenetic tree of Vap B Proteins.

The 3D coordinate files of the above cancer target proteins were downloaded from the Protein Data Bank (PDB) with PDB IDs of 3H87 and 5X3T for VapB2–VapC2 and VapB26–VapC26 respectively. Both the target proteins were analyzed via X-ray diffraction.

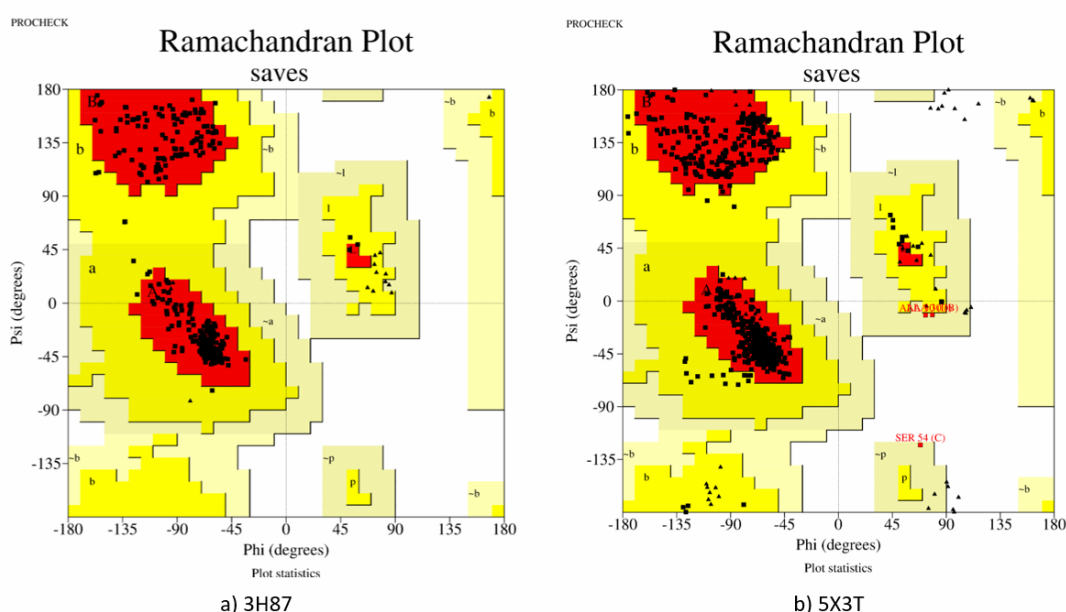


Figure 5: Ramachandran Plot revealing Protein structure prediction of VapBC proteins

The quality of the proteins was evaluated via Ramachandran plots via the SAVESv6.0 server (<https://saves.mbi.ucla>). SAVES v6.0 consists of a package of five programs. ERRAT scores of 96.7742% and 92.5247% validated the overall quality of the protein structure for VapB2–VapC2 and VapB26–VapC26, respectively. The PROCHECK analysis for VapB2–VapC2 revealed that 97.3% of residues fell in the most favored regions, 2.7% in the additional allowed regions, 0.0% in the generously allowed regions, and 0.0% in the disallowed regions. Similarly, PROCHECK analysis of the VapB26–VapC26 revealed that 95.4% of the residues fell in the most favored regions of the Ramachandran plot, 4.1% in the additional allowed regions, 0.4% in the generously allowed regions, and 0.0% in the disallowed regions. The Ramachandran plot calculations revealed that the above data obtained from other programs confirmed that the overall quality of the protein was good and could be used for further experiments (Figure 5).

3.6. Molecular docking

Protein-protein docking was done using ClusPro. The PDB files of the toxin and antitoxin were prepared using Discovery studio visualizer and were uploaded on ClusPro for docking.

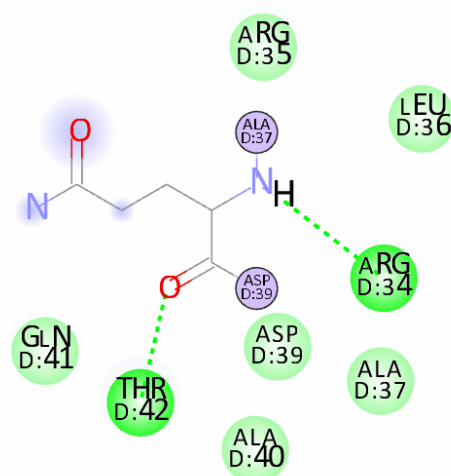


Figure 6: Elucidation of Protein-protein docking accomplished employing ClusPro.

The two-dimensional (2D) interaction map was generated using Discovery Studio Visualizer. A total of two hydrogen bonds were identified, represented in dark green, involving the amino acid residues ARG D:34 and THR D:42 as shown in Figure 6. Additionally, six van der Waals interactions were observed. This finding precisely reflects a core principle in biochemistry: hydrogen bonds (H-bonds) are crucial for protein-ligand complex stability, as they provide specific, directional interactions that contribute to strong binding (affinity) and maintain the complex's overall shape (structural integrity). More H-bonds generally mean better stability and binding, though water's role in displacing these bonds can add complexity to predicting net affinity (Tam et al., 2022). The presence of hydrogen bonds contributes significantly to the stability of the protein–ligand complex; hence, the identified interactions suggest good binding affinity and overall structural stability. Further, Based on validation through manual literature search and through comparison of two protein complexes from the SKEMPI dataset, DiffBond was able to identify intermolecular ionic bonds and hydrogen bonds with high precision and recall, and identify salt bridges with high precision. DiffBond predictions on bond existence were also strongly correlated with observations of Gibbs free energy change and electrostatic complementarity in mutational experiments (Tam et al., 2022). DiffBond can be recommended as a powerful tool for predicting and characterizing influential residues in protein-protein interactions, and its predictions can support research in mutational experiments and drug design (Donald et al., 2011; Tam et al., 2021; Tam et al., 2022).

3.7. Codon usage and expression feasibility in *E. coli*

For codon usage and expression feasibility, JCat was used. All VapB and VapC genes exhibited a CAI value of 1.0, which is significantly higher than the threshold value of 0.8, indicating excellent codon optimization and high expression feasibility in *E. coli*. The Codon Adaptation Index (CAI) and GC content of the selected VapB (antitoxin) and VapC (toxin) genes from *Mycobacterium tuberculosis* were analysed to evaluate their potential expression efficiency in *Escherichia coli* as shown in Table 3 and 4.

Table 3: CAI Scores and GC Content of Selected VapB Anti-Toxin genes with reference to *E. coli* Genome GC Composition.

S.NO.	Anti-toxin	CAI	GC Content (E coli)	GC Content (anti-toxin)
1	VapB1	1	50.74	54.45
2	VapB2	1	50.74	59.84
3	VapB3	1	50.74	55.32
4	VapB26	1	50.74	54.95
5	VapB28	1	50.74	54.74

The GC content of *E. coli* was approximately 50.73%, whereas the GC content of the antitoxin genes ranged from 54.43% to 59.81%, and the toxin genes ranged from 55.30% to 59.12%. These values are slightly higher than that of *E. coli*, but still within an acceptable range, suggesting no major GC bias that could hinder expression. Overall, the results suggest that both vapB and vapC genes are well-suited for heterologous expression in *E. coli*, with optimal codon usage and compatible GC content profiles.

Table 4: CAI Scores and GC Content of Selected VapC Anti-toxin Genes with Reference to *E. coli* Genome GC Composition.

S.NO.	Toxin	CAI	GC Content (E coli)	GC Content (anti-toxin)
1	VapC1	1	50.74	56.40
2	VapC2	1	50.74	56.74
3	VapC3	1	50.74	59.15
4	VapC26	1	50.74	55.34
5	VapC28	1	50.74	56.18

IV. Conclusion and Future Perspectives

The present *in-silico* study provides a comprehensive overview of the structural, evolutionary, and functional characteristics of VapBC toxin–antitoxin (TA) systems of *Mycobacterium tuberculosis*, with emphasis on their behavior in an *E. coli* expression. Sequence analysis was done to identify the conserved catalytic and regulatory motifs, reaffirming the functional importance of the PIN RNase domain in VapC toxins and the RHH/HTH DNA-binding motifs in VapB antitoxins. Phylogenetic reconstruction revealed clear evolutionary clustering and co-divergence of VapB and VapC partners, underscoring their long-standing functional interdependence. Structural modeling and validation further confirmed the robustness and stability of VapBC complexes, while protein–protein docking highlighted key interacting residues responsible for toxin neutralization. Codon optimization analysis demonstrated that *stcoli*, supporting future experimental efforts using heterologous systems. Taken together the studies (Dwivedi et al., 2008; Dwivedi et al., 2011), cooperatively, these findings strengthen the evidence that VapBC TA modules play essential roles in stress adaptation, persistence, and virulence in *M. tuberculosis*. Their involvement in growth regulation, RNase activity, and host-induced stress tolerance, VapBC systems represent promising targets for therapeutic intervention. Nevertheless, comprehensive approaches conjoining structural biology, genetics, transcriptomics, and *in-vivo* infection studies will be indispensable in view of completely understanding their physiological roles. The overall results gathered from the present study may provide new insights into establishing a strong platform for further experimental validation and thus aiding to develop new strategies to fight drug-resistant TB. Moreover, an unexplained question remains: What is the catalytic mechanism of the nucleases (principally from the VapC-like group), whose configurations were solved in the presence of only one metal ion?

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Data availability statement: The data used in the current study is available in the repository, Protein Data Bank.

Conflict of interest: Authors declare no conflict of interest.

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