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STRUCTURAL VARIATION AND TRANSITIONAL ANALYSIS OF *EMBB* RECEPTOR WITH ITS MUTANTS LEADING TO DRUG RESISTANCE IN TUBERCULOSIS

VAMIKA KARN[1], VARUN P. TALATI[1], SHASHANK S. KAMBLE[1] AND VIRUPAKSHA A. BASTIKAR[1]*

[1]Center for Computational Biology and Translational Research, Amity University Mumbai, Panvel, Mumbai 410 206, M.S., India

*Amity Institute of Biotechnology, Amity University Maharashtra, Mumbai - Pune Expressway Bhatan, Somathne, Panvel, Mumbai 410 206, M.S., India.

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Abstract– Ethambutol is an essential anti-tuberculosis drug widely used for the treatment of multidrug resistant tuberculosis (MDR-TB). However, resistance to ethambutol has been observed mainly due to mutations in probable arabinosyltransferase B (embB). In the current paper, we study the conformational changes occurring in probable arabinosyltransferase B due to mutations and their effect on the drug interactions. The mutants were modeled, energy minimized and docking was performed with ethambutol to study the interaction. On comparison with the wildtype, the rmsd of mutants were in the range of 0.5-0.65 and the average distance was 0.688. Amino acids Met306, Glu327 and Asp328 were observed to be involved in the ethambutol interaction network with the mutants having a disrupted network with these amino acids. High degree of mutations were observed between 306 and 497 amino acid positions with mutation at 306 being associated with high levels of ethambutol and multidrug resistance.

INTRODUCTION

Tuberculosis remains one of the most common causes of mortality due to infectious diseases, with significant medical, social, and economic implications (Shi *et al*., 2007). Ethambutol (EMB) [dextro-2,2′-(ethylenediimino) di-1-butanol], is an essential first-line anti-tubercular drug and is crucial in the therapy of drug-resistant tuberculosis (Sreevatsan *et al*., 1997). It primarily targets the probable arabinosyltransferase B. It prevents arabinogalactan and lipoarabinomannan from polymerizing and enhances the accumulation of D-arabinofuranosyl-P-decaprenol, an intermediate in arabinan biosynthesis (Wolucka *et al*., 1994; Zhang and Yew, 2009). It has been observed that 56.8% of the MDR-TB isolates displayed resistance to ethambutol with high degree of mutations found in the embB gene (Zhang *et al*., 2020). In this study, we observed that the arabinofuranosyltransferase central domain and arabinosyltransferase C-terminus harbors the mutations that cause the drug resistance to EMB. The identified mutations are S297A, M306I, M306L, M306V, D328G, D328Y, F330V, Y334H, G406A, G406C, G406D, Q497K, Q497R, G745D, D959A, M1000R, and D1024N.

The phenotypic resistance towards first line anti-tubercular drugs is associated with changes in the molecular targets (receptors) of these drugs within *M.tb* which eventually leads to the development of MDR-TB. These changes in *M.tb* proteins arises as a result of point mutations which alters its association with anti-tubercular drugs. When the amino acid residues of TB receptors undergo alteration (substitution, insertion, deletion), the interactions observed will be different as compared to wildtype. Subsequently, the mutated structures might show a drastic phenotypic change compared to its wildtype counterparts. In addition, these structures are highly unstable due to the presence of several strands than β-sheets/α-helices. As a consequence of the conformational changes of these mutants, the drugs

(*Associate Professor)

will not show the same activity although they may bind to the same active site. Since the structures of the mutants change or even if active sites change, first-line drugs will not interact in the similar way as they do in case of the wild type pathogen. Hence, the most common drugs used in the treatment of TB do not work against drug resistant strains of *M.tb*.

Since MDR tuberculosis comprise approximately 20.5% approximately of all TB cases worldwide, it is imperative to study and understand the structure of mutated molecular targets or receptors of these drugs so that effective inhibition approaches can be designed and developed against the same. Eventually, this may lead to better management of MDR TB patients and increase their life expectancy. This analysis provides a detailed evaluation of mutant conformations and structural changes in the receptor protein followed by how it interacts with the current approved drug.

MATERIALS AND METHODS

Hardware and software

The study was carried out on a desktop computer with a 3.60 GHz processor, 8 GB RAM, and 1 TB hard drive running in the Windows operating system. Information about the receptor was retrieved from the following databases: The Uni Prot database (Wang *et al.*, 2021) and the RCSB PDB database (Burley *et al.*, 2021).

The wild type of the selected receptor was used to create 17 mutant variants. The full-length structure of the wildtype and mutants were modeled by performing structure prediction using I-TASSER (Yang *et al.*, 2015; Yang and Zhang, 2015; Zheng *et al.*, 2021). YASARA server (Krieger *et al.*, 2009) was used for energy minimization of the modeled structures. For visualization, PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.) was used to calculate RMSD and deviation values between the wildtype and mutants. Further, to validate the minimized predicted structures, PDBsum (Laskowski *et al.*, 2018), ProSA (Sippl, 1993; Wiederstein and Sippl, 2007) analysis were performed.

METHODOLOGY

Protein Structure Prediction

The sequence for the probable arabinosyltransferase B was obtained from Uniprot. (Uni Prot Consortium, 2021) along with the mutations which confer resistance to the ethambutol in *M. tuberculosis.* Ab initio modelling was performed through the I-TASSER server for structure prediction. Among the models obtained, the one with the highest c-score was selected and its TM-score and RMSD values were noted.

Energy Minimization

The predicted structures were energy minimized to reduce the overall potential energy of the protein and to find the minimum energy conformation. The energy minimization was carried out using the YASARA energy minimization server where the results were saved in sce format and viewed in YASARA view. The start and end energy of the structure were noted and the minimized structures were saved in pdb format.

Protein Structure Validation

To validate the quality of the predicted structures, Protein Structure Analysis (ProSA) and PROCHECK were performed. ProSA is used for the refinement and validation of experimental protein structures whereas PROCHECK checks the stereochemical quality of a protein structure generating Ramachandran plots.

Structural Comparison

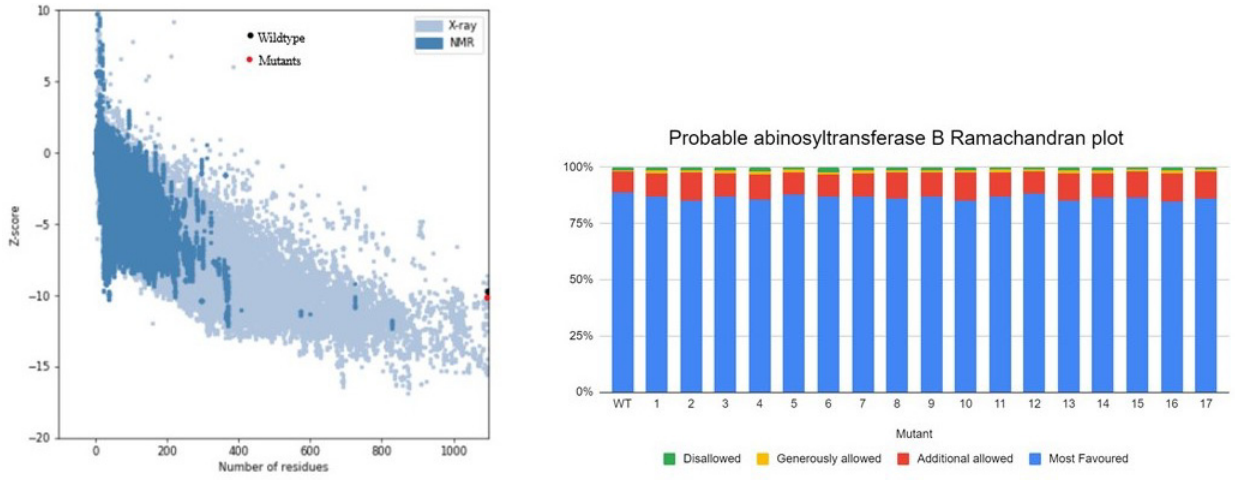
The mutant structures were compared to the wildtype on PyMOL to calculate their RMSD values and deviation values.

Docking

Docking of ethambutol drugs with the wildtype and mutants was performed using Autodockvina tools. The docking protocol was followed according to "AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading" (Trott and Olson, 2010).

RESULTS AND DISCUSSION

The structures obtained from I-TASSER had an average c-score of 0.57. All the mutants and wild-type structure predicted using the I-TASSER server were energy minimized with the help of the energy minimization server, YASARA. The energies of the wild type and mutant structures after energy minimization were in the range of -510934 kJ/mol to -499202.8 kJ/mol. The rmsd of the mutants of



A B

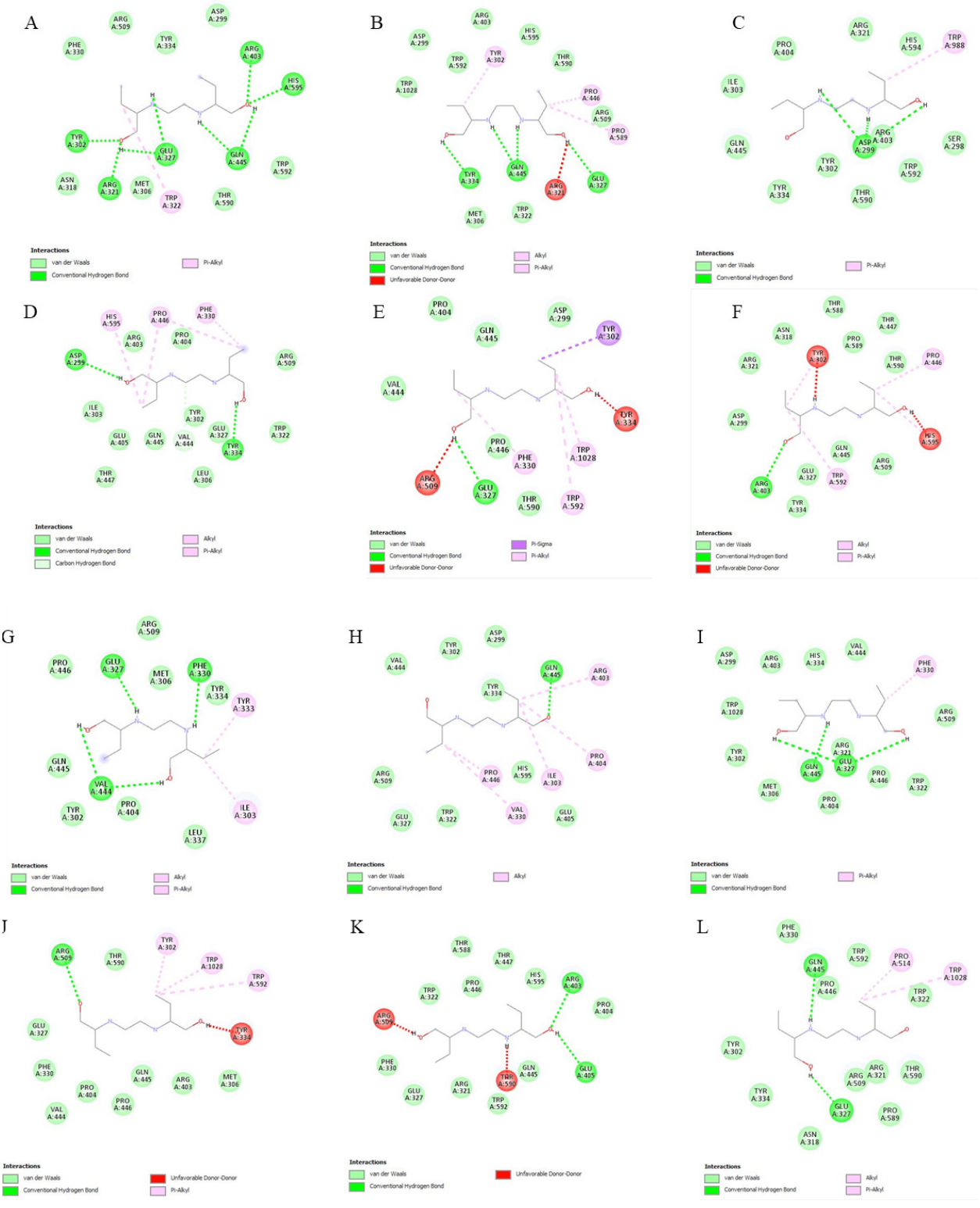
Fig. 1. (A) Plot for Z-score obtained from ProSA for the wildtype and mutants for probable arabinosyltransferase B. (B)Ramachandran plot generated for the wildtype and mutants (1) S297A, (2) M306I, (3) M306L, (4) M306V, (5) D328G, (6) D328Y, (7) F330V, (8) Y334H, (9) G406A, (10) G406C, (11) G406D, (12) Q497K, (13) Q497R, (14) G745D, (15) D959A, (16) M1000R, (17) D1024N of probable arabinosyltransferase B.

ethambutol receptor showed low values with the range of 0.5-0.65 with the highest of mutant 14 is 0.643. The average distance of the mutants was 0.688, among which mutant 6 and mutant 14 displayed the maximum distance of 2 and 1.4 respectively.

ProSA is a popular tool for detecting errors in 3D models of protein structures. It has a wide range of

Table 1. Interaction network of ethambutol observed with wildtype and mutants of embB receptor.

S. No.	Mutation	Interacting amino acids	Type of interaction
1.	Wildtype	Tyr302, Arg321, Glu327, Arg403, Gln445, His595; Trp322	Conventional hydrogen bond; Pi-alkyl
2.	S297A	Glu327, Tyr334, Gln445; Tyr302, Pro446, Pro589; Arg321	Conventional hydrogen bond; Pi-alkyl; Unfavorable donor-donor
3.	M306I	Asp299; Trp988	Conventional hydrogen bond; Pi-alkyl
4.	M306L	Asp299, Tyr334; Phe330, Pro446, His595	Conventional hydrogen bond; Pi-alkyl
5.	M306V	Glu327; Phe330, Trp592, Trp1028; Tyr334, Arg509; Tyr302	Conventional hydrogen bond; Pi-alkyl; Unfavorable donor-donor; Pi-sigma
6.	D328G	Arg403; Pro446, Trp592, Tyr302, His595	Conventional Hydrogen Bond; Pi-Alkyl; Unfavourable donor-donor
7.	D328Y	Glu327, Phe330, Val444; Ile303, Tyr333	Conventional Hydrogen Bond, Pi-Alkyl
8.	F330V	Gln445; Ile303, Val330, Arg403, Pro404, Pro446	Conventional Hydrogen Bond, Pi-Alkyl
9.	Y334H	Glu327, Gln445,; Phe330	Conventional Hydrogen Bond, Pi-Alkyl
10.	G406A	Arg509; Tyr302, Trp592, Trp1028; Tyr334	Conventional Hydrogen Bond, Pi-Alkyl, Unfavourable donor-donor
11.	G406C	Arg403, Glu405; Arg509, Thr590	Conventional Hydrogen Bond, Unfavourable donor-donor
12.	G406D	Glu327, Gln445; Pro514, Trp1028	Conventional Hydrogen Bond, Pi-Alkyl
13.	Q497A	Glu327, Gln445; Tyr302, Pro446, Pro589	Conventional Hydrogen Bond, Pi-Alkyl
14.	Q497R	Glu327, Tyr334, Gln445; Trp592	Conventional Hydrogen Bond, Pi-Alkyl
15.	G745D	Asp299; Trp592	Conventional Hydrogen Bond, Pi-Sigma
16.	D959A	Asp299, Arg403; Trp988; Trp1028	Conventional Hydrogen Bond, Pi-Alkyl, Pi-Sigma, Unfavourable acceptor-acceptor
17.	M1000R	Gln445, Tyr302	Conventional Hydrogen Bond, Pi-Sigma
18.	D1024M	Asp299, Arg403, Gln445; Tyr302; Trp592, Trp1028	Conventional Hydrogen Bond, Pi-Sigma, Pi-Alkyl


A
B
C
D
E
F
G
H
I
J
K
L
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Alkyl
Pi-Alkyl
Pi-Sigma

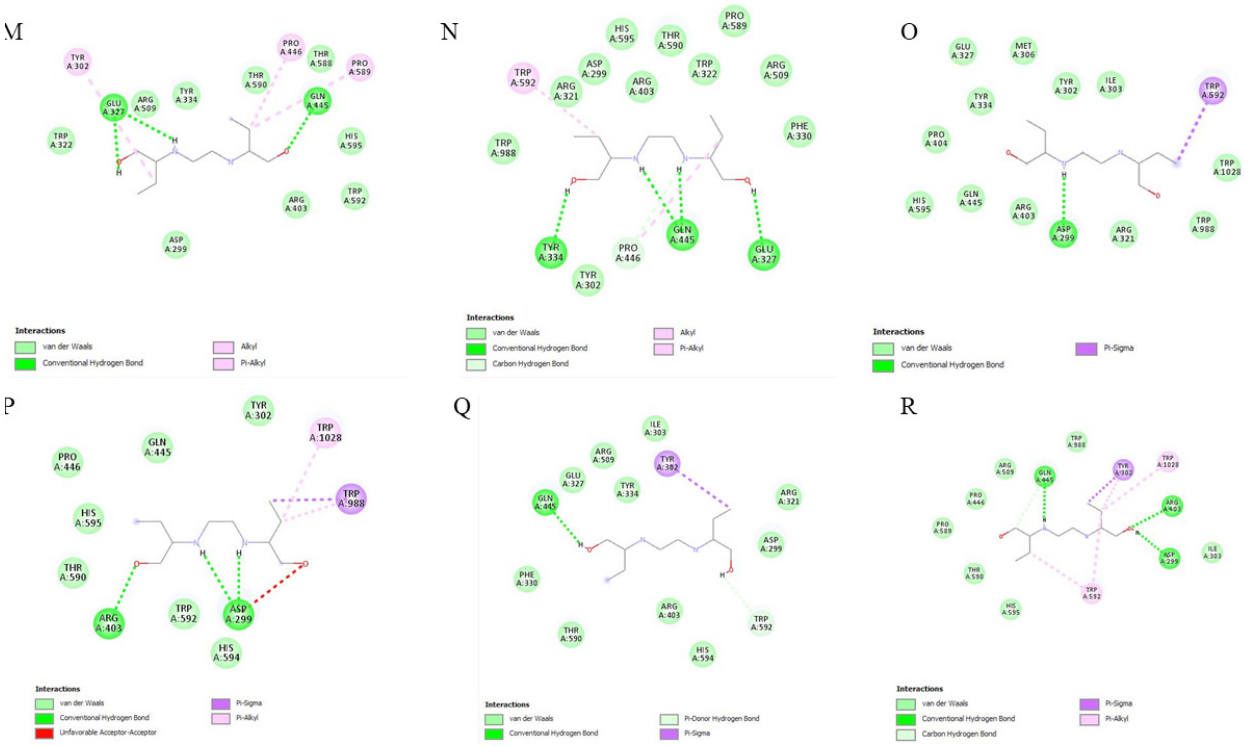


Fig. 2. Interactions of ethambutol with probable arabinosyltransferase B (A) wildtype, (B) S297A, (C) M306I, (D) M306L, (E) M306V, (F) D328G, (G) D328Y, (H) F330V, (I) Y334H, (J) G406A, (K) G406C, (L) G406D, (M) Q497K, (N) Q497R, (O) G745D, (P) D959A, (Q) M1000R, and (R) D1024.

applications, from error detection in experimentally obtained structures to theoretical models and protein construction (Sippl, 1993; Wiederstein and Sippl, 2007). The z-score represents the overall quality of the protein structure in this case. The wildtype and mutant average z-scores obtained from ProSA for overall quality assessment via NMR and X-ray crystallography were -9.69 and -9.80, respectively. The resulting data revealed that the overall structure quality was acceptable as the z-score falls under the X-Ray crystallography region. As a result, the modeled structures can be used for further research.

The data generated from the Ramachandran plot for all 17 mutants and the wild-type is shown in the graph above. The PDBsum database was used for this. The quality and stability of the structure were assessed based on its location. The core regions have the best (blue) combinations, as well as the highest number of points. The additional allowed regions (red) can be found near or unrelated to the core regions; however, they have fewer data points than the core regions. The generously permitted regions (yellow) go beyond the permitted regions. The remainder of the areas are prohibited (green) (Laskowski *et al.*, 2018). The Ramachandran plot of all the predicted structures showed above 85% residues in the most favored region with mutant 12 having the highest percentage (88.4). Thus, demonstrating good quality structures.

Docking

The wildtype shows strong interactions with ethambutol through conventional hydrogen bonds with Tyr302, Arg321, Glu327, Arg403, Gln445, and His595 along with pi-alkyl bonds with Trp322. Three mutational hotspot at position Met306, Gly406, and Gln497 have been identified apart from other mutations. Met306 is implicated in non-polar interactions with residues Tyr302 and Glu327, wherein Glu327 interacts with ethambutol (Zhang *et al.*, 2020). Therefore, mutations at position 306 (mutants M306I, M306L, M306V) alter the interaction network, impacting EMB binding. Similarly, upon mutation at Gln497 (mutants Q497K, Q497R), the Glu327-ethambutol interaction is disrupted as the mutation prevents the interaction with Glu328 (Zhang *et al.*, 2020). Therefore, mutations at Asp328 (mutants D328G, D328Y) will impede the Glu327-ethambutol interaction.

Mutations at Gly406 are assumed to cause steric hindrance which further leads to conformational changes at the active site (Zhang *et al.*, 2020).

High degree of mutations were observed in the region between 306 and 497 with mutation at the 306 being the most prevalent followed by embB406 and 497 (Zhao *et al*., 2015). embB306 mutants have been observed in the development of high-level ethambutol resistance. It has also been reported that mutations at 306 position might provide a predisposition for the development of isoniazid or rifampicin resistance. Therefore, these mutants may have a higher chance in evolving into MDR and XDR strains during therapy (Safi *et al*., 2008).

CONCLUSION

This analysis offers a thorough assessment of mutant conformations and structural alterations in Probable arabinosyltransferase B (embB) along with structure quality validation. Since the structure of the receptor changes in a mutation, so does its interaction with the drug. Thus, we have shown the interactions of embB mutants with ethambutol. Furthermore, comparative analysis of interactions of mutants with wildtype embB was also performed to infer the changes in them. Our study shows that all the residues in the vicinity of 5.0 Angstrom depict an interaction network of all amino acids responsible for ethambutol activity. This allows us to understand the unfavorable interactions for the embB - ethambutol complex. Moreover, it provides data to develop new analogs that circumvent these unwanted interactions thus possibly showing activity on drug resistant MTB.

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