Clin. Lab. 2026;72:1-7

ORIGINAL ARTICLE

Comparative Analysis of Host-Pathogen Protein-Protein Interactions between Human and Various Strains of *Mycobacterium Tuberculosis*

Devvret Verma [1], Bhavya Mudgal [1], Neema Tufchi [1], Madhulika E. Prasad [1], Kumud Pant [1], Ashish Thapliyal [2], Debasis Mitra [2], Shreesh Gupta [3], Rokayya Sami [4], Uguru Hilary [5], Roua S. Baty [6], Roqayah H. Kadi [7], Ashwaq M. Al-Nazawi [8, 9], Nada Alqadri [10]

[1] *Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India*
[2] *Department of Microbiology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India*
[3] *Wecapsulate Pharma, Kanodli, Dehradun, Uttarakhand, India*
[4] *Department of Food Science and Nutrition, College of Sciences, Taif University, Taif, Saudi Arabia*
[5] *Department of Agricultural Engineering, Delta State University of Science and Technology, Ozoro, Nigeria*
[6] *Department of Biotechnology, Faculty of Science, Taif University, Taif, Saudi Arabia*
[7] *Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia*
[8] *Department of Public Health, College of Nursing and Health Sciences, Jazan University, Jazan, Saudi Arabia*
[9] *Laboratory Department, Jazan University Hospital, Jazan University, Jazan, Saudi Arabia*
[10] *Department of Biology, Turabah University College, Taif University, Taif, Saudi Arabia*

SUMMARY

Background: **Tuberculosis remains a substantial health threat globally, despite decades having elapsed since the identification of its causative agent, *Mycobacterium tuberculosis.* Approximately 35% of the global population is sub-clinically infected, leading as one of the primary causes of human mortality. The increased prevalence of drug-resistant strains of Mtb necessitates identification of important drug targets. Therefore, the aim of the study was to comparatively analyze the protein-protein interactions between the host and the pathogen (*Mycobacterium tuberculosis*) to uncover the conserved molecular mechanisms of infection, providing insight into strain-specific variations.**

Methods: **One of the major problems is the diverse spectrum of diseases caused by different Mtb. To date, most research has their attention on a specific pathogenic strain. Therefore, to screen common and effective drug targets of different strains, we compared the protein-protein interactions of four virulent strains (H37Rv, CDC1551, CAS/NITR204, and Erdman) and one a virulent strain (H37Ra) of Mtb with its human host. Here, the interolog method was adopted to identify the biomolecular-interactions between Mtb and its human host.**

Results: **As a result, an interaction network has been developed, and the target has been screened through multiple parameters, such as the highest interacting partners, virulent factors, subcellular localization, and predicted protein interactions.**

Conclusions: **This study substantially resulted in the identification of potential drug targets, ATP synthase subunit alpha and gamma, and chaperone proteins DNAK and HTPG.**

(Clin. Lab. 2026;72:1-7. DOI: 10.7754/Clin.Lab.2025.241235)

Correspondence:
Devvret Verma
Department of Biotechnology
Graphic Era (Deemed to be University)
Dehradun
248002 Uttarakhand
India
Email: devvret@geu.ac.in

Rokayya Sami
Department of Food Science and Nutrition
College of Sciences
Taif University
Taif 21944
Saudi Arabia
P.O. box 11099
Email: rokayya.d@tu.edu.sa

Manuscript accepted March 20, 2025

Supplementary Data

An overview of the potential drug targets identified

After studying the host-pathogen protein-protein interactions (HP-PPIs), the ATP synthase (ATPA & ATPG) and Chaperon proteins (DNAK & HTPG) were found to be present in all the studied strains, which suggests that these proteins can be a potential drug target and can also fight against the problem of the diverse pattern of infection through the bacterium *Mycobacterium tuberculosis* (*Mtb*). Various steps of filtration of HP-PPIs, like subcellular localization, virulence factor, function and role in pathways, etc., were studied to sort out potential drug target of *Mtb*.

ATP synthase as a potential drug target

ATP synthase is the membrane-bound ion channel that usually controls the movement of ATP and ions through membranes [1]. F0F1 ATP synthase performs hydrolase activity and was considered a potential drug target in this study. The mycobacterial ATP synthase complex has been studied and it has turned out to be a successful target for the anti-tubercular drug Bedaquline. It has been previously reported that the F-ATPase subunit alpha of *Mtb* has a unique C-terminal extension of 36 amino acid residues [2]. In the alignment study of amino acid sequences of ATPA by clustalW, a unique amino acid region, i.e. 514-TGGGSVVPDEHVEALDEDKLAKEAVKVKKP APKKKK-549 (according to *Mtb* numbering), was revealed, which is absent in any other known eukaryotic or prokaryotic alpha subunits of ATPase (Figure S1). The secondary structure analysis predicted this region (514-549) of *Mtb* ATPA protein as a beta turn (Figure S2). Likewise, studies on the F1FOATPase subunit (γ) gamma considered it a drug target as it showed lower homology with F1FOATPase γ subunit of *Escherichia coli* and human mitochondrial, i.e. a sequence similarity of 37% and 25%, respectively. The region of 14 amino acid residues 165-TDNGEDQRSDSGEG-178 (according to *Mtb* UniProt ID P9WPU8 numbering) was found to be unique, as it was absent in other prokaryotic or eukaryotic γ subunits (Figure S3) [3].

Besides this, there are several structural differences when compared with the human orthologue, and because of these differences in mycobacterium and human mitochondrial ATP synthase, the subunit alpha and gamma can be considered as a potential drug target. In the future, these drug targets can be used for docking analyses; phytochemicals and drug repurposing can help us to develop new drugs against the proposed target [4].

Protein chaperones as a potential drug target

Protein chaperones are important for all domains of life for preventing and resolving protein misfolding occurring during the translation process and during the proteotoxic stress. DNAK belongs to the heat shock family 70 protein and is widely distributed in both prokaryotic and eukaryotic cells. The function of DNAK is well studied for *E. coli*, yet its functions for other bacteria have not been studied extensively. Mycobacterial DNAK regulates the heat shock response by interacting with HspR C terminal tail and relieves repression of chaperone genes. The heat shock response helps in the survival of the cells that are exposed to a sudden increase in ambient temperature [5-7]. The heat-shock response is a ubiquitous adaptive pathway involved in the survival of cells exposed to a sudden increase in ambient temperature. It is characterized by global transcriptional changes including elevated expression of a set of highly-conserved heat-shock proteins. DNAK is regulated by cofactors, nucleotide exchange factor GrpE, and J proteins. In mycobacterium, two types of J proteins are present, DnaJ1 and DnaJ2, which activate the ATPase activity of DNAK differently. *Mtb* DNAK and their eukaryotic homologs Hsp104/ Hsp70 are ATP-powered chaperones that restore toxic protein aggregates to a native folded state. These chaperones play an important role in establishing the infection by *Mtb* [8].

Another studied prokaryotic 90-kDa molecular chaperone, HtpG protein or high temperature protein G, belongs to the class of heat shock protein and is about 40% similar to its eukaryotic counterparts [9]. HtpG or Hsp90 functions are similar to DNAK. It is not very well characterized unlike its eukaryotic counterparts [10]. Hsp90 is also involved in drawing out immune responses [11]. Studies show that it is responsible for T-cell immunity [12]. Chaperone peptide's processing and MHC-1 presentation is shown to be increased with the help of hsp90 and hsp70 (DNAK) [13]. Inhibition of the function of HSP90 provides a new approach to fight against drug resistance and overcome the virulence of a pathogen. It was previously reported that HtpG affects the dormant phase of *Mtb* [14].

References:

1. Cole LA. Biology of Life: Biochemistry, Physiology and Philosophy. Academic Press 2016. https://doi.org/10.1016/C2015-0-05632-5

2. Lakshmanan M, Xavier AS. Bedaquiline - The first ATP synthase inhibitor against multi drug resistant tuberculosis. J Young Pharm 2013;5(4):112-5. (PMID: 24563587)

3. Priya R, Biuković G, Manimekalai MSS, et al. Solution structure of subunit γ (γ 1-204) of the Mtb F-ATP synthase and the unique loop of γ 165-178, representing a novel TB drug target. J Bioenerg Biomembr 2013;45(1-2):121-9. https://www.doi.org/10.1007/s10863-012-9486-4

4. Devvret, Pant K, Thapliyal A, Tufchi N. In Silico Docking analysis of Mtb potential targets AftB and EmbA with selected phytochemicals. Int J Pharm Res Technol 2017;2:15-22. https://ijprt.org/index.php/pub/article/view/79

5. Hartl FU. Molecular chaperones in cellular protein folding. Nature 1996;381(6583):571-9. https://doi.org/10.1038/381571a0

6. Stewart GR, Wernisch L, Stabler R, et al. Dissection of the heat-shock response in Mycobacterium tuberculosis using mutants and microarrays. Microbiology (Reading) 2002;148(10):3129-38. (PMID: 12368446)

7. Lupoli TJ, Vaubourgeix J, Burns-Huang K, Gold B. Targeting the proteostasis network for mycobacterial drug discovery. ACS Infect Dis 2018;4(4):478-98. https://doi.org/10.1021%2Facsinfecdis.7b00231

8. Stewart GR, Robertson BD, Young DB. Analysis of the function of mycobacterial DnaJ proteins by overexpression and microarray profiling. Tuberculosis (Edinb) 2004;84(3,4):180-7. https://doi.org/10.1016/j.tube.2003.12.009

9. Bardwell JC, Craig EA. Eukaryotic Mr 83,000 heat shock protein has a homologue in Escherichia coli. Proc Natl Acad Sci U S A 1987;84(15):5177-81. https://dx.doi.org/10.1073%2Fpnas.84.15.5177

10. Genest O, Hoskins JR, Camberg JL, Doyle SM, Wickner S. Heat shock protein 90 from Escherichia coli collaborates with the DnaK chaperone system in client protein remodeling. Proc Natl Acad Sci U S A 2011;108(20):8206-11. https://dx.doi.org/10.1073%2Fpnas.1104703108

11. Qamra R, Mande SC, Coates ARM, Henderson B. The unusual chaperonins of Mycobacterium tuberculosis. Tuberculosis (Edinb) 2005;85(5,6):385-94. https://doi.org/10.1016/j.tube.2005.08.014

12. Quintana FJ, Carmi P, Mor F, Cohen IR. Inhibition of adjuvant-induced arthritis by DNA vaccination with the 70-kd or the 90-kd human heat-shock protein: immune cross-regulation with the 60-kd heat-shock protein. Arthritis Rheum 2004;50(11):3712-20. https://doi.org/10.1002/art.20635

13. Tobian AAR, Harding CV, Canaday DH. Mycobacterium tuberculosis heat shock fusion protein enhances class I MHC cross-processing and-presentation by B lymphocytes. J Immunol 2005; 174(9):5209-14. (PMID: 15843516)

14. Neckers L, Tatu U. Molecular chaperones in pathogen virulence: emerging new targets for therapy. Cell Host Microbe 2008;4(6): 519-27. https://doi.org/10.1016%2Fj.chom.2008.10.011

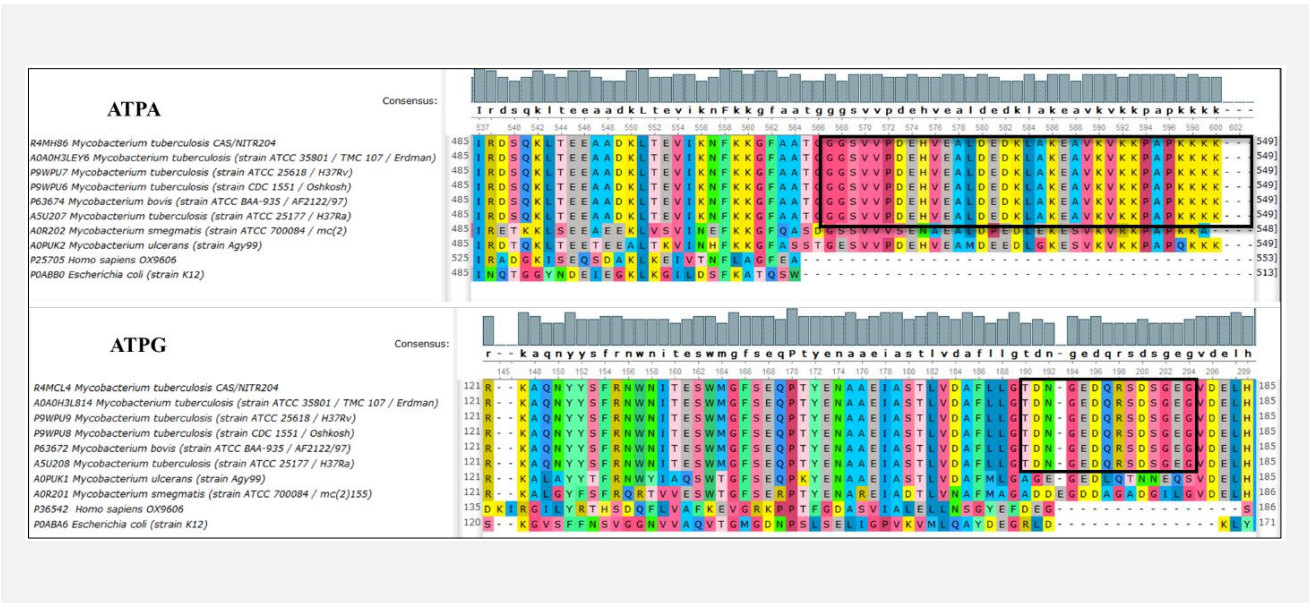

Figure S1. Clustal Omega sequence alignment of ATPase subunit alpha and gamma sequences of different strains of *Mycobacterium* along with sequences of *E. coli* and human.

The region in black box (514 - 549 of ATPA and 165 - 178 of ATPG) is uniquely present in *Mtb* strains. The alignment is obtained through Unipro UGENE tool.

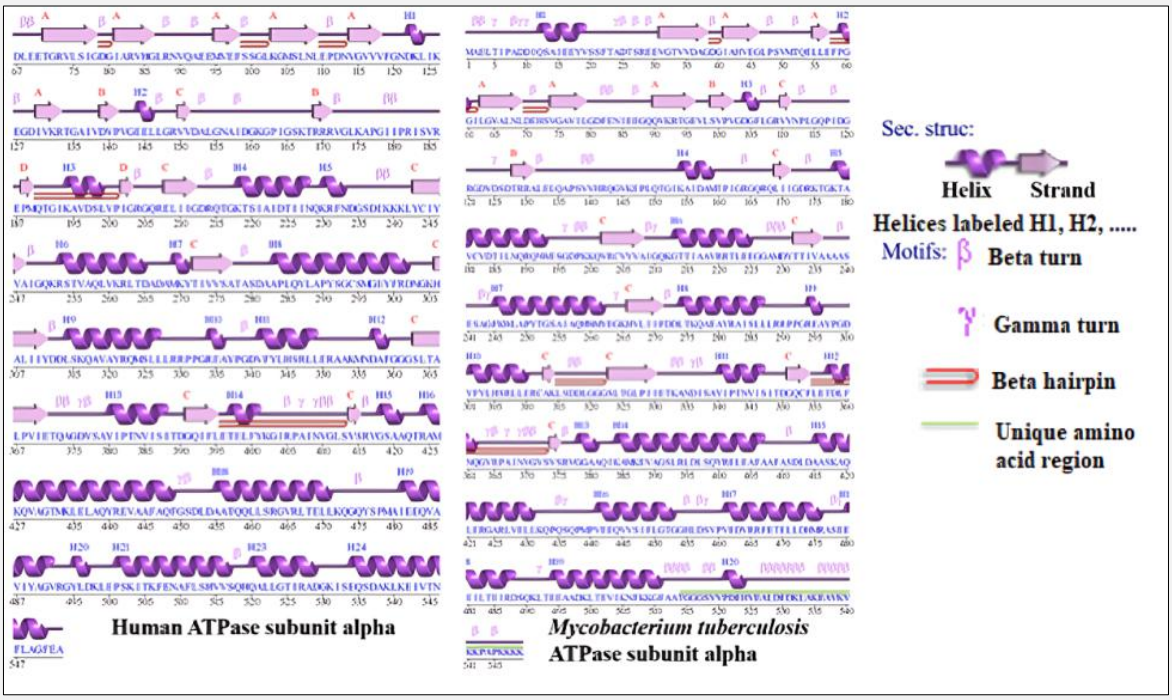

Figure S2. Secondary structure analysis through PDBsum showing the unique region of ATPase subunit alpha in *Mtb*; a structural comparison with human ATPase subunit alpha.

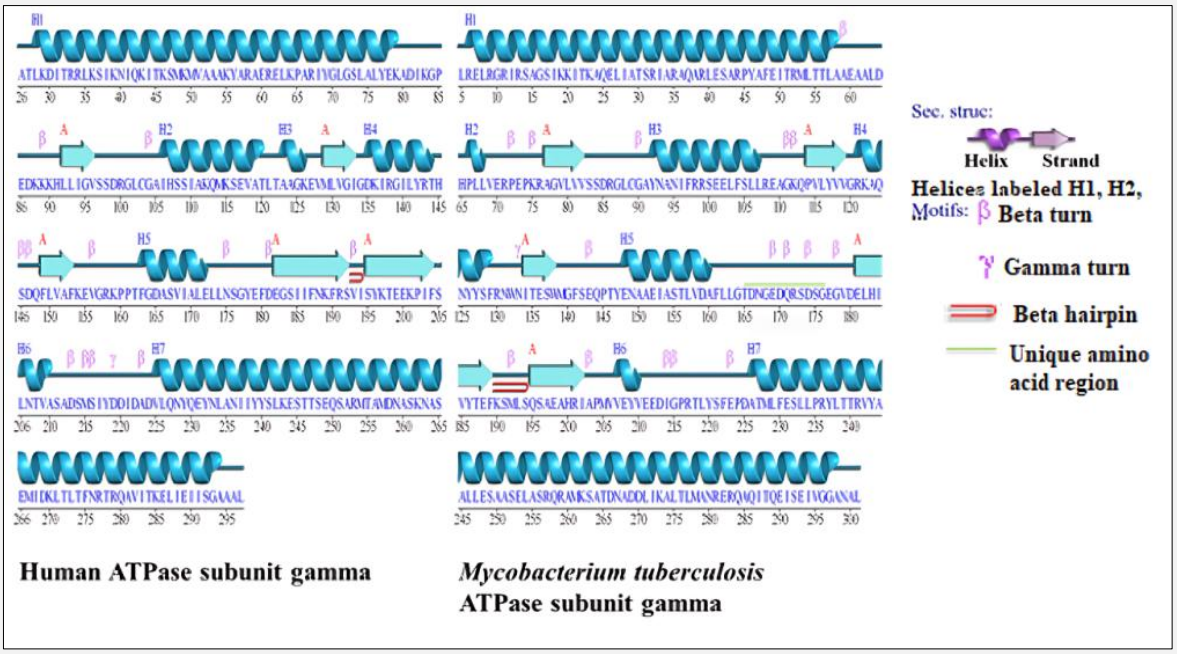

Figure S3. Secondary structure analysis through PDBsum showing the unique region of ATPase subunit gamma in *Mtb*; a structural comparison with human ATPase subunit alpha.