RES-388

APCCMI2025 BANGKOK

Identification of Key Genetic Targets for Tuberculosis Diagnosis: Advancing Molecular Detection within a One Health Approach

Siti Kurniawati[1,3*], Nanda Ayu Cindyas Putri[2], Wahyu Nur Laili Fajri[3], Slamet Riyanto[4], Indah Amalia Amri[1]

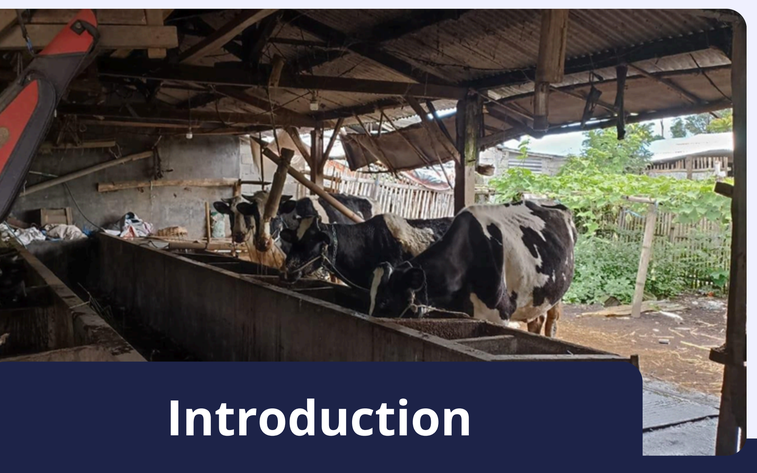
[1]Veterinary Microbiology and Immunology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya

[2]Faculty of Veterinary Medicine, Universitas Brawijaya

[3] Animal Diagnostic Diseases Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya

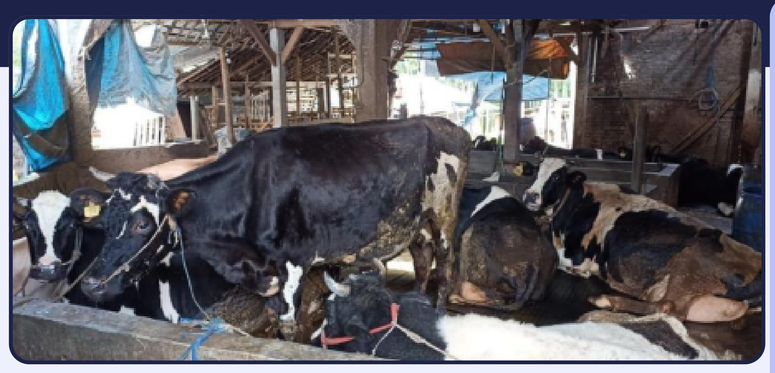
[4] Department of Clinical Microbiology, Faculty of Medicine, Universitas Brawijaya

*Corresponding Author: sitikurniawati9@ub.ac.id

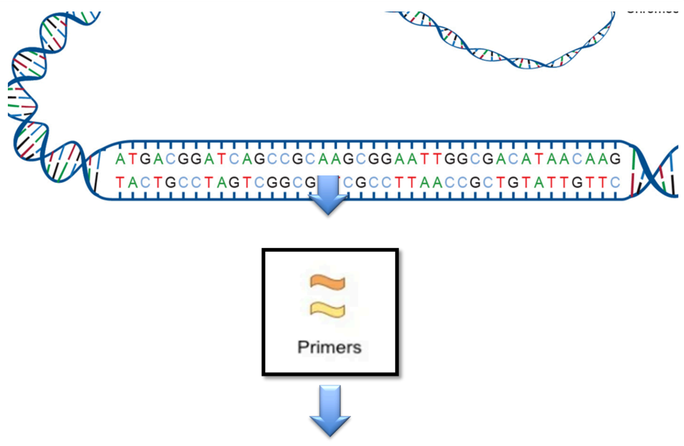


Introduction

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* complex (MTBC) and remains a significant zoonotic threat with impact on both human and animal health. Beyond M. tuberculosis in humans, species such as M. bovis and M. caprae are responsible for bovine tuberculosis, contributing to economic losses in livestock and serving as important sources of zoonotic transmission. Despite the global burden of TB, current diagnostic methods frequently lack sufficient speed, specificity, and cross-species applicability, thereby limiting their effectiveness for early detection and control in both medical and veterinary contexts. To address this challenge, the present study focused on identifying and characterizing reliable genetic targets for the development of molecular diagnostics. Four essential genes *leuS* (leucyl-tRNA synthetase), *ku* (DNA repair protein), *gyrB* (DNA gyrase subunit B), and *rpoB* (RNA polymerase β-subunit) were selected based on their critical biological functions, high conservation across MTBC species, and established diagnostic relevance.



Method



Result

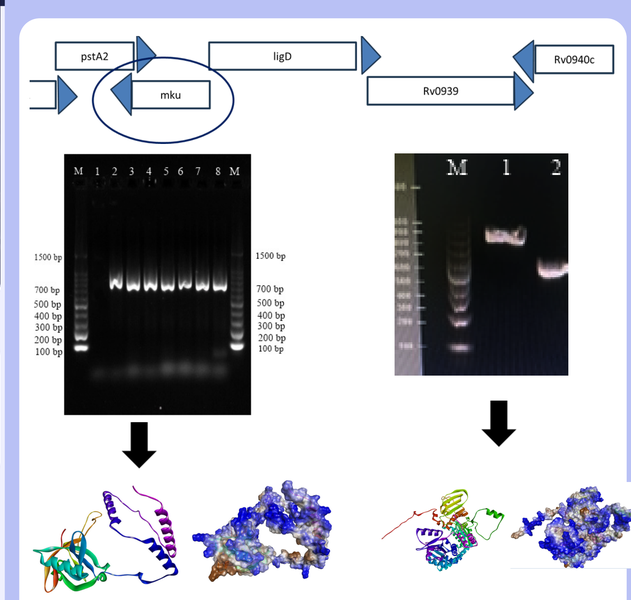


Figure 1. detection *ku* gene and protein

Amplification of the ku gene produced a specific band at the expected size (700 bp). Structural modeling of the Ku protein demonstrated a conserved domain associated with DNA repair activity, reinforcing its potential as a stable diagnostic marker

Figure 2. detection *gyrB* gene and protein

The *gyrB* gene showed clear bands (600 bp) in lane 2 with 100% identity to *M. tuberculosis* H37Rv. Its protein domains linked to DNA supercoiling make it a stable and valuable marker for identifying MTBC species.

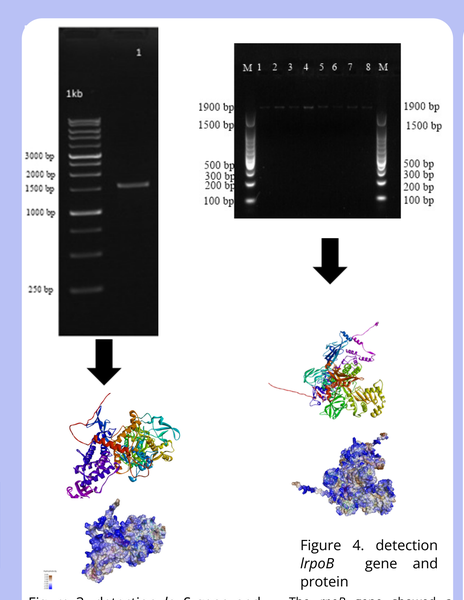
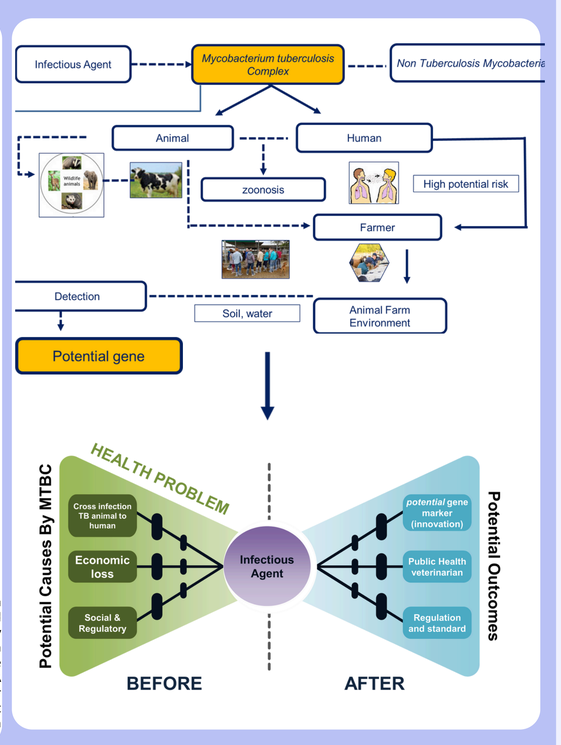


Figure 3. detection *leuS* gene and protein

The leuS gene produced clear bands (1481bp) and matched 100% with *M. tuberculosis* H37Rv. Its protein structure showed a conserved active site, confirming its vital role in protein synthesis and reliability as a TB marker.

Figure 4. detection *lrpoB* gene and protein

The *rpoB* gene showed a clear band at the expected size, 1956bp with the H37Rv reference. Its protein structure confirmed the conserved β-subunit of RNA polymerase, a key marker not only for TB detection but also for rifampicin resistance.



Discussion

All four gene targets (*leuS, ku, gyrB,* and *rpoB*) successfully produced specific amplicons, with no bands in *Non-Tuberculous Mycobacteria* (NTM), confirming high specificity (Zhao et al., 2016). Sequencing showed 100% identity with M. tuberculosis H37Rv, validating diagnostic accuracy (Cole et al., 1998). These genes are highly conserved in the *Mycobacterium tuberculosis* complex, which includes zoonotic members such as *M. bovis* and *M. caprae* that cause tuberculosis in cattle and other animals (Jagielski et al., 2014; WHO, 2021). The use of *rpoB* and *gyrB* is especially relevant since they are associated with drug resistance and differentiation among MTBC species (Böttger, 2011; Ramasoota et al., 2021). Thus, *leuS, ku, gyrB,* and *rpoB* represent reliable molecular markers not only for human TB but also for animal TB diagnosis, strengthening control strategies at the human–animal interface within a One Health framework (OIE, 2022).

The One Health approach is crucial, as TB remains a zoonotic disease that requires cross-sector and interdisciplinary collaboration linking human, animal, and environmental health at both local and international levels to achieve sustainable health outcomes (WHO, 2021; FAO-OIE-WHO, 2019). These findings highlight the potential of *leuS, ku, gyrB*, and *rpoB* as reliable genetic markers for rapid and accurate TB detection within the One Health approach.

Conclusion

This study successfully identified and verified l*euS, ku, gyr*B, and *rpoB* as potential molecular targets for tuberculosis diagnosis. Their high specificity could be developed for rapid diagnostics, such as nucleic acid lateral flow assays. These findings advance the development of cross-sectoral TB diagnostic tools in alignment with One Health strategies. A broader evaluation involving multiple geographic regions is recommended

References

- Böttger EC. The spectrum of rpoB mutations in Mycobacterium tuberculosis and their clinical significance. Clin Microbiol Infect. 2011;17(10):1522–7.
- Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature. 1998;393(6685):537–44.
- FAO, OIE, WHO. Taking a Multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries. Paris: WHO Press; 2019.
- Jagielski T, Van Ingen J, Rastogi N, Dziadek J, Mazur PK, Bielecki J. Current methods in the molecular typing of Mycobacterium tuberculosis and other mycobacteria. Biomed Res Int. 2014;2014:645802.
- Ramasoota P, Jaksuwan R, Kaewprasert O, Moolphate S, Kaewthamasorn M, Payungporn S. Molecular markers associated with rifampicin resistance in Mycobacterium tuberculosis. Antibiotics. 2021;10(8):952.
- Waters WR, Palmer MV, Buddle BM, Vordermeier HM. Bovine tuberculosis vaccine research: Historical perspectives and recent advances. Vaccine. 2012;30(16):2611–22.
- World Health Organization. Global tuberculosis report 2021. Geneva: WHO; 2021.
- World Organisation for Animal Health (OIE/WOAH). Terrestrial Manual: Infection with Mycobacterium tuberculosis complex. 2022.
- Zhao Y, Xu S, Wang L, Chin DP, Wang S, Jiang G, et al. National survey of drug-resistant tuberculosis in China. N Engl J Med. 2016;366(23):2161–70.