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学位論文内容の要旨
Abstract of the dissertation

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The title of the doctoral dissertation

Development and Evaluation of Diagnostic Methods for Bovine
Tuberculosis and Johne's Disease

（牛結核およびヨーネ病の診断法の開発と評価）

Bovine tuberculosis (bTB) and Johne's disease (JD) remain major challenges to sustainable livestock production worldwide because of their economic burden, zoonotic potential, and long-term environmental persistence. Effective control of these diseases relies on early, accurate, and field-applicable diagnostic tools. This dissertation aimed to improve both serological and molecular diagnostic approaches for mycobacterial infections by addressing current limitations in antigen production and pathogen detection.

The first part of this study evaluated *Mycobacterium smegmatis* as an alternative host for recombinant antigen production to address the low diagnostic sensitivity of bTB serodiagnosis. Recombinant proteins expressed in *Escherichia coli* often lack post-translational modifications present in native *Mycobacterium bovis* antigens, which may compromise diagnostic performance. Ten candidate antigens were expressed individually in *E. coli* and *M. smegmatis* and assessed by enzyme-linked immunosorbent assay (ELISA) using plasma samples from interferon-gamma release assay (IGRA)-negative (n = 30) and IGRA-positive cattle (n = 46). Several antigens expressed in *M. smegmatis* demonstrated better concordance with IGRA compared with their *E. coli* counterparts. Notably, LprA expressed in *M. smegmatis* showed significant

concordance with IGRA, comparable to that of the well-established antigen MPB70. Furthermore, a multiple logistic regression model incorporating eight antigens derived from both expression hosts markedly improved discrimination between IGRA-positive and IGRA-negative animals, achieving near-perfect classification performance. These results suggest that *M. smegmatis* is a promising host for producing recombinant antigens with improved serological relevance and that multi-antigen strategies may enhance bTB serodiagnosis.

The second part described the development of a multiplex loop-mediated isothermal amplification (LAMP) assay coupled with DNA chromatography for the simultaneous detection of *M. bovis* and *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agents of bTB and Johne's disease, respectively. Probit regression analysis using purified genomic DNA estimated the 95% limits of detection at 240 fg per reaction for *M. bovis* BCG and 13 fg per reaction for MAP. In spiked samples, the assay detected *M. bovis* BCG at 1.1×10^3 CFU/mL in milk and 3.5×10^4 CFU in 10% fecal suspensions, and MAP at 3.4×10^2 CFU/mL in milk and 1.5×10^3 CFU in 10% fecal suspensions. The assay showed high analytical specificity against 24 non-tuberculous mycobacterial species and three enteric bacteria, with minor cross-reactivity observed in a small number of genetically related species and no false-positive results in negative controls.

Overall, this dissertation demonstrates that the use of *M. smegmatis* as an alternative expression host to improve the diagnostic performance of recombinant antigens, together with the development of rapid, affordable molecular diagnostics, can complement existing diagnostic frameworks and provide practical approaches for the surveillance and control of mycobacterial diseases in livestock.