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WARALEE TORSUWAN : DIFFERENTIATION OF MYCOBACTERIAL SPECIES BY AMPLIFICATION OF 16S-23S rDNA SPACER COMPARISON TO AMPLIFICATION OF OTHER GENES AND IMPROVEMENT OF THE DETECTION METHOD. THESIS ADVISORS : PRASIT PALITTAPONGARNPIM, M.D., SOMSAK RIENTHONG, M.Sc., MONGKOL KUNAKORN, M.D. 132 p. ISBN 974-663-077-6

Tuberculosis, which is caused by *Mycobacterium tuberculosis*, is a major cause of mortality in humans. Since the spread of human immunodeficiency virus, the rate of infection by *M. tuberculosis* and *Mycobacterium* other than tuberculosis (MOTT) has been increasing. Therefore, other methods for mycobacterial species identification that are more rapid and accurate than the conventional biochemical method are required. In this study, the polymerase chain reaction and restriction enzyme analysis (PCR-REA) of 16S-23S rDNA spacer was compared to PCR-REA of *hsp65* and *dnaJ* gene. Moreover, simple methods for identifying mycobacterial species using probe specific to 16S-23S rDNA spacer were studied.

It was found that PCR-REA of 16S-23S rDNA spacer could discriminate mycobacterial species as well as PCR-REA of *hsp65*, though PCR-REA of *hsp65* had more intraspecies variation. PCR-REA of *dnaJ* had lower discriminating capacity. In the development of simple methods, PCR-ELISA and microtiter plate sandwich hybridization was tested. The first method had a sensitivity such that it could detect DNA concentration as low as 2 pg, while the second method was more sensitive with the lowest detected DNA concentration of 100 fg. Moreover, dot blot hybridization could identify some *M. tuberculosis* from lymph node samples and reverse dot blot hybridization could also identify mycobacterial species from clinical culture. Therefore, these methods will be useful for rapid and accurate method of mycobacterial species identification.