Thesis Title A Study on the 16S rRNA Gene of *Mycobacterium tuberculosis*

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Abstract

This study was an attempt to investigate 16S rRNA gene of mycobacterium species. The aim of this investigation was to explore the possibility to employ this gene for development of a rapid identification method for *M. tuberculosis* and some other pathogenic mycobacteria. The gene (~1.5 kb) of *M. tuberculosis* was amplified from 1 reference strain, 8 Thai isolates and 4 other species of *M. tuberculosis* complex(*M. tuberculosis, M. bovis, M. africanum*, and *M. microti*) using polymerase chain reaction (PCR). The amplified products were subsequently subjected to direct sequencing. These sequence data together with the previously published ones indicated that the coding region of this gene was highly conserved. Oligonucleotide primers have been designed from both coding and noncoding regions of the gene and used as an identification approach in multiplex PCR. *M. kansasii* and *M. avium* which also cause pulmonary infection were included in this assay. The results indicated that these species could be distinguished from each other by specific amplification patterns with the exception *of M. tuberculosis, M. bovis* and *M. bovis* BCG which possessed their own common patterns. Furthermore, the 16S rRNA gene of *M. tuberculosis* was radioactively labeled by PCR and used as a hybridization probe to examine the copy number of rRNA operon in mycobacterium species. This probe was hybridized to mycobacterial DNAs which were immobilized on nitrocellulose or nylon membrane by dot blotting and Southern techniques. Dot blot hybridization revealed that rapid - growing mycobacteria possessed two times more copies of rRNA gene than slow - growing mycobacteria. Southern analysis of Pst I - digested DNA supported this possibility and also suggested that slow - growing mycobacteria contained one copy of rRNA gene, and thus one rRNA operon, whereas rapid - growing species contained two copies of this gene, and thus two rRNA operon, in their chromosome, as previously reported by other investigators.