Thesis Title	Identification of *Mycobacterium* species by Amplification of 16S-23S ribosomal DNA spacer and Reverse dot-blot hybridization
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ABSTRACT

Members of the genus *Mycobacterium* are wide-spread in nature, ranging from harmless inhabitants of water and soil to the agents of diseases. Tuberculosis is still a major health problem in many parts of the world. The problem is compounded by the coincidental increase of large outbreaks of multidrug resistance, and the increasing number of cases co-infected with HIV. The increase in mycobacterial diseases has stimulated the development of more rapid and efficient methods of diagnosis. Recently molecular techniques such as the polymerase chain reaction (PCR) have been developed in several microbiological laboratories for the rapid differentiation of mycobacterial species.

The purpose of this study was to develop a method to identify *Mycobacterium* species based on the amplification of the spacer sequences between 16S and 23S rDNA gene. 192 isolates of mycobacteria belonging to 20 species of the genus *Mycobacterium* were studied. Firstly, *Mycobacterium* species were identified by PCR combining with restriction enzymes analysis. After that the species identification was done by PCR followed by

hybridization with species-specific oligonucleotide probes selected from the spacers using dot-blot and reverse dot-blot hybridization techniques. 8 oligonucleotide probes MTB-24, MV-21, MI-23, MG-24, MK-I23, MK-II22, MS-I23, and MS-IV21 were specific to *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. gordonae*, *M. kansasii* I, *M. kansasii* II, *M. scrofulaceum* I and II, and *M. scrofulaceum* IV respectively. Whereas MC-23 and MF-23 showed non-specific hybridization. All tested isolates of *M. tuberculosis* complex, *M. avium*, and *M. kansasii* II could hybridized with MTB-24, MV-21, and MK-II22 respectively. MI-23, MG-24, and MK-I23 could not hybridize with all tested isolates, with 1/18, 3/40, and 2/10 not being hybridized to each probe respectively. MS-I23 and MS-IV 21 gave inconsistent signals which were difficult to observe. These 2 probes were, therefore, still not suitable to be used to identify clinical isolates.

The differentiation of the *Mycobacterium* species by the species-specific oligonucleotide probe hybridization to the amplified 16S-23S rDNA spacer is a rapid more than biochemical tests, reliable method and is suitable for use in identifying *Mycobacterium* species directly from clinical specimens.