CHAPTER I

INTRODUCTION

Tuberculosis is the world's second commonest cause of death from infectious disease after HIV/AIDS epidemic. There was an estimation of 8-9 million new cases of tuberculosis in 2000 [1]. Nearly 2 million people died of tuberculosis, with a global case fatality rate of 23% but reaching >50% in some African countries due to high rate of coexisting human immunodeficiency virus (HIV) infection. The actual global prevalence of *M. tuberculosis* infection is 32%, corresponding to approximately 1.9 billion people [2]. If control of tuberculosis is not further strengthened in the future, the World Health Organization (WHO) estimates that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will bacome sick, and another 35 million people will die from infections [1].

Although *Mycobacterium tuberculosis* complex (MTC) remains responsible for the majority of *Mycobacterium* infections worldwide, many nontuberculous mycobacteria (NTM) are also of medical relevance [3, 4], in particular, as a consequence of the AIDS epidemic. Major causative agents of nontuberculous mycobacterial infections are *M. avium, M. intracellulare, M. chelonae, M. abscessus, M. fortuitum, M. kansasii,* and *M. xenopi* [3-6]. With these increases in the incidences of tuberculosis and other infections caused by NTM, there is a growing need for more specific identification, particularly since infections caused by different *Mycobacterium* species often require different treatment regimens.

The traditional method of *Mycobacterium* species identification relies upon the phenotypic characteristics of biochemical testing, pigment production, growth characteristics, and colonial morphology [7] . Culture has long been considered the gold standard and the development of automated liquid culture system has reduced the time for growth confirmation compared to solid media [8-10]. The extent of biochemical testing depends on the ease of characterization and biochemical activity of the *Mycobacterium* under investigation. Biochemical tests are simple to execute, require minimal equipment,

and generally accurately differentiate between the more common species. However, they are time-consuming and present a delay to final identification due to long incubation times. They require experience in interpretation and are limited by subjectivity and low specificity [11, 12] . With almost 100 currently established species, a number that continues to rise, biochemical algorithms become too complex, which results in an inherent bias towards the identification of more familiar species of mycobacteria. Biochemical algorithms usually include on average 15 to 20 species only. Phenotypic methods are still used in some laboratories to identify NTM despite their acknowledged difficulty [13].

Chromatographic techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and gas-liquid chromatography (GLC) can be used to differentiate most species of mycobacteria, but require special equipment and standardization of growth and working condition [14-16]. Several other molecular genetic methods have also been reported. These include amplification of species-specific sequences [17-19], PCR amplification and restriction enzyme analysis (PCR-REA) [20-24], hybridization with species-specific oligonuleotides probes with or without prior DNA amplification [25-27], and nucleic acid sequence determination [3, 23, 28-33].

Identification by use of nucleic acid probes (Gen-Probe, Inc., San Diego, Calif.) is a rapid and widely procedure but requires a well-growth culture and testing with several probes and covers only a narrow range of mycobacterial species [34, 35]. Recently a new DNA probe kit (INNO-LiPA Mycobacteria; Innogenetics, Ghent, Belgium), is available only in Europe at present. It is based on the reverse hybridization principle. The main advantage of the kit is that a range of several species can be identified by a simple PCR assay [12, 36]. However, the high cost of these assays prohibited large-scale usage in most clinical laboratories.

The 16S rRNA gene is the most widely accepted gene used for bacterial identification. It has contributed to the discovery of new species of the *Mycobacterium* genus, and it continues to serve as an important tool as an alternative to phenotypic identification methods [11, 30]. Multiplex PCR method, described by Wilton and Cousins [37], and confirmed by Kulski, et al. [38], to detect and identify members of the genus *Mycobacterium, M. tuberculosis, M. avium*, and *M. intracellulare*, is based on 16S

rRNA gene. The region A of 16S rRNA gene is known to be variable enough for species identification of mycobacteria and oligonucleotide probes specific at genus or species level have been designed by several investigators [31, 39]. Multiplex PCR of this DNA target followed by reverse dot blot hybridization using multiple species-specific probes is a promising approach for rapid detection and identification of mycobacterial species. The developed in-house method should provide a low-cost alternative to commercial probe methods for detection and identification of mycobacterial species.