# CHAPTER I
# INTRODUCTION

Mycobacteria are still a significant cause of morbidity and mortality of the world population. Currently, almost 100 species of mycobacteria [1] have been identified, many of which are associated with human disease. Despite the abundance of mycobacteria species, the following few species or groups cause most human infection: *Mycobacteria tuberculosis, M. leprae* and nontuberculous mycobacteria (NTM) [2].

*M. tuberculosis* infection remains the most successful human pathogen worldwide, and more than one-third of the world's population is exposed to the infection every year[3, 4]. In 2002, the World Health Organization (WHO) estimated that there were 8 million new cases of tuberculosis (TB), causing 2.5 million deaths per year[5]. Regions with the highest incidence of disease were Southeast Asia, Sub-Saharan Africa, and Eastern Europe. In 2004, there were 9 million new TB cases and approximately 2 million TB deaths[6]. In 2006, there were an estimated 9.2 million new cases of TB. This is an increase from 2005, reflecting population growth in Asia, Africa and Europe. These statistics show that TB remains a major global health problem[7].

While *M. tuberculosis* complex strains are still responsible for the majority of *Mycobacterium* infections worldwide, opportunistic infections due to NTM have been on the increase, mainly as a consequence of the AIDS epidemic. Among the mycobacterial species often implicated in NTM infections are *M. avium*, *M. intracellulare*, *M. kansasii, M. fortuitum,* and *M. scrofulaceum* [5, 8-10]. NTM can cause a wide variety of infections including pulmonary, lymphatic, skin and soft tissue, skeletal, and catheter-related infections[11].

Before the acquired immunodeficiency syndrome (AIDS) epidemics, the pulmonary infections caused by NTM were found predominantly in males in the sixth decade of life. Most patients have predisposing lung conditions or work under conditions where they were exposed to contaminated dusts. The major pulmonary

pathogens include *M. avium* complex (MAC), *M. kansasii,* and rapidly growing mycobacteria (RGM) such as *M. fortuitum*[12]. *M. scrofulaceum* is found to be the causative agent of cervical lymphadenitis in children[13]. In a previous report on human immunodeficiency virus-negative patients from Switzerland, from 1983 to 1988, the incidence of tuberculosis declined from 16.2 to 13.2 per 100,000 inhabitants while the incidence of mycobacteriosis increased from 0.4 to 0.9 per 100,000 inhabitants. In England, a shift occurred between 1970, when the ratio of 1 NTM was isolated for every 60 *M. tuberculosis* isolates, and increase to the ratio of 1 NTM for every 6 *M. tuberculosis* isolates in 1992 [14].

Since the AIDS epidemic, the picture of NTM infections has been radically changed. The 25-50% of patients with AIDS in United State and Europe are infection with NTM, mostly by MAC[15-17]. In Thailand, the first patient with NTM infection caused by *M. kansasii* was reported in 1968[18]. Since then, there were several reports of NTM infections caused by *M. scrofulaceum*, *M. gordonae*, MAC, *M. chelonae*, *M. fortuitum*, *M. szulgai*, *M. smegmatis*, *M. marinum* and other RGM[19-21]. In 2006, King Chulalongkorn Memorial Hospital reported that the MAC was the predominant species (48.5%), followed by 19.4% of *M. kansasii*, and 16.4% of RGM in HIV patients [22]. In 2004, the study of Naowrat Kunyanone [23] had shown that most of mycobacteria isolated from HIV-infection patient in Chiang Mai province were NTM. They were *M. avium* 25%, followed by *M. intracellulare* 18%, *M. scrofulaceum* 13% and *M. kansasii* 0.03% of NTM.

The rapidly spreading human immunodeficiency virus epidemic in many parts of the world will future increase the number of HIV-related cases of tuberculosis as well as the number of nontuberculous mycobacteria infections. The rapid and accurate identification of clinically significant mycobacteria species is necessary for optimal medical and public health interventions [24]. The result of traditional methods for identification of closely related *Mycobacterium* species by growth characteristics (pigment, growth rate, colonial morphology) and biochemical tests are often difficult to interpret, even for some very common species. The tests are cumbersome and time-consuming, require expertise, lack sensitivity and reproducibility[25]. Rapid biochemical methods, such as high performance liquid chromatography (HPLC) of

mycolic acids and gas chromatography (GC) of fatty acids are available only in specialized laboratories [26].

The rapid identification of mycobacteria to the species level is recommended in clinical laboratories to ensure accurate diagnosis and effective therapy. This has stimulated the development in recent years of a number of rapid and more accurate identification tools based on molecular technology [27]. In the present, the Multiplex PCR is one choice for detection and identification of mycobacteria due to it is not cumbersome and time-consuming. However, in the previous study, the Multiplex PCR is identified a few species of mycobacteria.

The ITS is approximately 270 to 360 bp but varies in size from species to species. It is considered to be suitable target for primer with which additional phylogenetic information can be derived[28]. Furthermore, the ITS is suitable for differentiating species of mycobacteria and potentially can be used to distinguish clinically relevant species[29].

All the previous report, the species of mycobacteria is frequently involved in human disease as *M. avium, M. intracellulare, M. kansasii, M. fortuitum, M. scrofulaceum* and *M. tuberculosis* complex. So this study, the Multiplex PCR was established for identification of 6 species of mycobacteria simultaneously by Multiplex PCR assay based on the 16S-23S rRNA gene internal transcribed spacers (ITS).

**Objectives of the study**

1. To develop the detection rate of mycobacteria and identification of *M. avium, M. intracellularae, M. kansasii, M. fortuitum, M. scrofulaceum* and *M. tuberculosis* complex by using single tube Multiplex PCR.
2. To evaluate sensitivity of Single-tube Multiplex PCR in detection and identification of mycobacteria.
3. To compare the identification capability of clinical isolate mycobacteria between Single-tube Multiplex PCR with PCR-REA and PNB screening test.