Chapter I

Introduction

About 1,700 million people ,or one third of the world's population are, or have been,infected with *Mycobacterium tuberculosis*. It is estimated that, in 1990 , there were totally 8 million new cases of tuberculosis in developing and industrialized countries :7.6 million (95%) in the former and 400,000 (5%) in the latter. (It is estimated that tuberculosis caused 2.9 million death from a single pathogen in the world).

There is a striking difference in the patients who suffer from tuberculosis between developing and industrialized coutries due to the differences in the pathogenesis of the disease in these countries. In industrialized countries, tuberculosis is mainly seen in the elderly. In developing countries the risk of infection remains high and tuberculosis afflicts nearly all age groups (Kochi, A.1991).

In Thailand, tuberculosis is one of the major public health problems. It has been not only rated as the fourth leading cause of death in the country, but also is the first leading cause of death by infectious agent (Public Health Ministry,1989).

The main components of tuberculosis control programmes are case case-finding finding, treatment and BCG vaccination.

Although BCG prevents childhood tuberculosis, it has been reported that in nine field trials including North American Indians,

Chicago infants, Georgia school children, Illinois children, Puerto Rico general population, Georgia and Alabama population, British school children, India (Madanapalle) and Haiti population which were studied in protective efficacy of BCG vaccination, with 95% confidence intervals, the protective effect was seen to vary from none at all to 80%. Unfortunately, the trials,even collectively, did not reveal what caused the differences in protection observed (ten Dam,1984).

At present, it has been accepted that case-finding and treatment of tuberculosis are the most effective ways for disease control.

The diagnosis of tuberculosis is still a public health problem and problematic. The definitive diagnosis of tuberculosis is to demonstrate viable tubercle bacilli in an appropriate clinical specimen. The acid-fast staining, which is very rapid, has low and variable sensitivity (Boyd, 1975 ; Burdash, 1976 and Lipsky, 1984). The finding of chest X-rays, results of tuberculin skin test, symptoms, signs and the history of tuberculosis contact can aid in making the diagnosis but are still not specific (Gordin, 1989). Serological methods have been developed from times to times, some gave promising results but some failed to give encouraging data. The DNA probe method, radiometric method and PCR method are complicate, requiring sophisticated instruments and reagents which are not generally available. In addition, they are costly for each test and required skilling techniques (Daniel,1988). Thus, the culture method is more definitive and sensitive. At present,the definitive diagnosis of this disease is still based on the results of acid fast staining and the culture for *M. tuberculosis*.

Routine work of microbiology is done by stianing acid fast bacilli (AFB) and culturing in solid media such as Lowenstein-Jensen medium (L-J medium). This isolation method is very useful for specimens with numerous tubercle bacilli, particularly sputum. Thus, The culture of this organism from other specimens with much fewer tubercle bacilli such as pleural effusion, ascites and cerebrospinal fluid (CSF) have often been found to be negative. In order to overcome the problem solid media can accommodate for only small amount of specimen., many studies reported that the isolation of tubercle bacilli in such specimens should be performed by using liquid media such as Kirchner medium (Mitchison,1983) and Middle-brook 7H9 broth (Martin,1989) in which large amount of liquid specimen can be added.

The objective of this thesis is firstly, to investigate the use of liquid media for the isalation of tubercle bacilli from liquid specimen with especially low amount of organisms. Secondly, to develope the appropriate liquid media for isolation of *Mycobacterium tuberculosis* from pleural effusion, ascites and CSF and to compare the isolation rate and cost of the developed liquid media with the commercial liquid media and also with the solid media (L-J medium). If the developed liquid media has the efficacy closed to commercial liquid media but has lower cost in production, it should be very useful for routine work in the isolation and identification of *M.tuberculosis* performed in hospitals especially the public ones.