Thesis Title	DNA Fingerprinting of *Mycobacterium tuberculosis* Thai Isolates
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Date of Graduation 6 May B.E. 2540 (1997)

ABSTRACT

Nowadays, tuberculosis is still a health problem throughout the world. The situations of AIDS pandemic and increasing multidrug-resistant *Mycobacterium tuberculosis* have evolved causing tremendous control problems of such disease. Developments of rapid diagnosis, an identification of the infectious source and the tracing of individuals in contact with the infected persons are important aspects of limiting the dissemination of tuberculosis. DNA fingerprinting of *M. tuberculosis* was the one of molecular approach that used for epidemiological studies of tuberculosis. Moreover, it has advantages in study the distribution of *M. tuberculosis* in every country worldwide, indicating the incidence of cross-contamination of specimens that cause misdiagnosis. The DNA fingerprinting by Southern blot-hybridization technique, now is in use around the world. It is recommended in the standardized

protocol to use restriction enzyme *Pvu*II and hybridized with IS*6110* probe.

In this study, 113 strains of *M. tuberculosis* clinical isolates from 96 patients were tested. DNA was extracted from cells by using physical rupture technique. Southern blot-hybridization was done by using standard methods. The results showed identical patterns within strains isolated from either same or different specimens from the same patient within 5 days. Ninety-six strains from different patients represented 69 different patterns of 1 to 20 hybridized bands. None of strain contained no IS*6110* was found. Sixty-nine isolates could be divided into 5 major groups according to pattern types and number of hybridized bands; single bander, few bands that produced 2 to 5 hybridized bands, heterogeneous group that produced more than 5 hybridized bands and showed the unique patterns, and the last two groups were the group of isolates that produced very similar patterns to one another, the Beijing family, firstly found in Beijing, China and the Nonthaburi group that reported firstly in Thailand. The percentage of each group was 26, 16.6, 18.8, 18.8 and 19.8, respectively. No relationships between ages of patients or site of disease and the types of patterns were observed. In addition, the correlation of drug susceptibility profile and pattern types were not found. Excluding the single banders, clusters were found more frequently in Beijing family and Nonthaburi group than others.

The epidemiological relationship among the single banders, by using IS*6110* as probe, could not be implied. Although six

different locations of hybridized band were found in this group, the additional probes were required. This study was also performed to assess the ability of the KS5, in-house DNA fragment, in differentiation among those single banders. The results showed ten different patterns with ≥ 3 hybridized-bands. However the discriminatory ability of KS5 was lower than the widely used probe(s), DR and/or PGRS. Moreover the results also indicated that KS5 carried IS*6110*.

This study also examined the discriminatory ability of KS5 among *M. tuberculosis* complex strains by using Southern blot-hybridization technique. By hybridized to *Pst*I-digested genomic DNA, the KS5 could differentiate *M. bovis* BCG from *M. tuberculosis* strains. *M. bovis* BCG strains could produce two type of patterns, one hybridized band at 8.5 kb and two hybridized bands at 17.9 and 8.5 kb whereas *M. tuberculosis* produced different type of patterns of more than 3 hybridized bands to *M. bovis* BCG. Therefore, the advantage of KS5 is differentiation of suspected cases of patients infected with *M. bovis* BCG or *M. tuberculosis*.