Thesis Title Mycobacterium tuberculosis : A Study on Deoxyribonucleic Acid (DNA) and Construction of DNA Libraries

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Date of Graduation 28 February B.E. 2533 (1990)

## ABSTRACT

Tuberculosis is a disease which is still considered to be a major global health problem, especially in developing countries. This disease is also prevalent among Thai people. In spite of the considerable efforts that have been made to control the disease in the last three decades, the reduction of the global incidence was not obviously observed. There are several problems encountered in controlling this disease. One of them is the difficulty in performing diagnosis of the mycobacterial infection. The current diagnostic procedures are lacking their sensitivities. This study is an attempt to develop a diagnostic method using deoxyribonucleic acid (DNA)-probe technology. Mycobacterial DNAs were isolated from the genomes of Mycobacterium tuberculosis by two comparative methods : the enzymatic lytic and the modified physically rupturing procedures. The DNAs obtained were initially checked whether they contained a plasmid that might determine the drug-resistant property. The result was negative.

To create recombinant DNA probes for mycobacterial identification, two genomic DNA libraries of M. tuberculosis were constructed in pBR322 vector by inserting the mycobacterial DNA fragments into the BamHI or PstI site of the plasmid and transforming them into Escherichia coli K-12 SF8. About 5,000 and 12,000 colonies obtained from the cloned BamHI and PstI libraries were found to contain recombinant plasmids with detectable fragments of the DNA inserted. Screenings for colonies containing recombinant plasmids potential to be used as the DNA probes were performed by using radioactively($^{32}P$) labeled total mycobacterial -genomic-DNA. Two colonies were selected and the recombinant DNA probes (pWR6 and pWR9) were isolated to evaluate their sensitivities and specificities in comparison with the total genomic DNA probes. Both of the recombinant and the total genomic DNA probes had high specificities in hybridization with the target DNAs from M. tuberculosis. However, their sensitivies were still too low to be used in a diagnostic application. Southern blot hybridization of restriction fragments of mycobacterial genomic DNA were employed by using the recombinant DNA probes. The result showed patterns of multiple restriction-DNA fragments hybridized with the two probes. It is most likely that the two DNA probes detected genomic repetitive DNA sequences and the cloned DNA fragments might be parts of these sequences.