Thesis Title Detection and Identification of *Mycobacterium tuberculosis* by Using DNA Probes

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ABSTRACT

Tuberculosis is an ancient disease and still a major global health problem. It is estimated that annually 3 millions people worldwide were died of this disease. Development of rapid and sensitive laboratory method for diagnosis of tuberculosis is a significant part in the attempt to control this disease. New technique in molecular biology are potential to be applied in this development. The results presented in this thesis were part of the continuous research work that applies molecular biology techniques for laboratory diagnosis of tuberculosis.

In this study, 5 mycobacterial DNA fragments (named as KS1, KS2, KS3, KS4 and KS5) were isolated from two recombinant plasmids, pWR6 and pWR9, previously cloned in our laboratory, for testing as DNA probes. Specificities of the

5 DNA probes were firstly evaluated by Southern blot hybridization using 11 DNA samples isolated from *M. tuberculosis* complex and 10 DNA samples isolated from *M. tuberculosis* obtained from patients. All the DNA probes, except KS3, could strongly hybridize to these DNA samples. However, only the KS4 and the KS5 DNA probes which showed multiple bands of hybridizations were selected for further testing in Southern blot hybridizations with DNA samples of 9 other species of mycobacteria, 12 species of bacteria and 2 species of yeasts. The two DNA probes showed very high specificities in hybridizations with DNA samples of *M. tuberculosis* complex but very weakly cross hybridization with DNA samples of *M. aurum*, *M. neolactis* and *P. aeruginosa*.

Sensitivities of the KS4 and the KS5 DNA probes in the detection of *M. tuberculosis* DNA were evaluated by dot blot hybridization with serially diluted mycobacterial DNA. The minimal amounts of DNA detected by the KS4 and the KS5 DNA probes were 100 pg and 1,000 pg (approximately 2.5×10^4 and 2.5×10^5 genomes), respectively.

It is difficult to lyse mycobacterial cells in the process of DNA isolation. Four methods of cell lysis and DNA isolation including modified physical rupture, enzymatic lysis, chemical lysis and boiling procedure were also tested by using decontaminated sputum samples containing known numbers of *M. tuberculosis* cells. Efficiencies of these methods were compared in dot blot hybridizations using the KS4 and the KS5 DNA probes. The modified physical rupture

method was found to be better than the other methods in isolation of the mycobacterial DNA and it was therefore used for isolation of mycobacterial DNA from patients' sputum samples in the follwing experiment.

Detection of the mycobacteria, specifically in *M. tuberculosis* complex, in 259 sputum specimens was carried out by dot blot hybridization evaluated using the KS4 and the KS5 DNA probes and the results were analysis in comparison with that obtained by the conventional methods. The samples were classified into three groups : positive (62 samples), suspected (146 samples) and negative (51 samples) tuberculosis based on the results of clinical diagnosis and routine laboratory examinations. The KS5 and the KS5 DNA probes had sensitivities at 32.2% (20/62) and 43.5% (27/62) and specificities at 98% (50/51) and 92.1% (47/51), respectively. The positive predictive values of the KS4 and the KS5 DNA probes were 95.2% and 87.1% and the negative predictive values were 54.3% and 57.3%. When the results of using the DNA probes were combined, the sensitivity and specificity of detection were 51.6% (32/62) and 92.1% (47/51), and the positive predictive and negative predictive values were 88.8% and 61%, respectively. In the group with suspected tuberculosis, approximately 24% (35/146) of the specimens were positive by dot blot hybridization using the two DNA probes while approximately 14% (21/146) were positive by acid-fast staining. The sensitivities of mycobacterial detection in sputum specimens by using the DNA hybri-

dization technique were lower than that of conventional methods in the group with patients of definite diagnosis of tuberculosis. This problem results from low efficiency of method used for isolation of the mycobacterial DNA in combination with low sensitivity of the DNA hybridization technique using DNA probes.

An application of using the KS5 DNA probe for identification of *M.bovis* BCG isolated from a patient with severe combined immunodeficiency syndrome (SCIDS) in Southern blot hybridization was demonstrated. The potential use of polymerase chain reaction (PCR) technique for detection of mycobacterial DNA was also presented in Appendix.