

## Abstract

In Taiwan, tuberculosis remains an important threat to public health, and multidrug-resistant *Mycobacterium tuberculosis* is an emerging problem. One of the most important measures to control the prevalence of tuberculosis is strain typing of *M. tuberculosis*. The data of strain typing is helpful in surveillance of the disease and providing knowledge of transmission of the organism in the community. Several techniques are now rather commonly used for typing of *M. tuberculosis*, including IS6110-restriction fragment length polymorphism analysis, the standard method for genotyping of *M. tuberculosis*, spoligotyping, variable-number tandem repeats (VNTR) typing, and mycobacterial interspersed repetitive units (MIRU) typing. The VNTR and MIRU methods are based on the fact that there are variable numbers of tandem repeats among different strains of *M. tuberculosis*. The purpose of the present project is to develop an automated MIRU method.

### Materials and Methods

Test organisms included 493 *M. tuberculosis* isolates from 203 patients collected between 2002 and 2003 at the Department of Pathology, National Cheng Kung University Hospital. Twelve targets were selected for MIRU analysis. Multiplex polymerase chain reaction (PCR) assays were performed to amplify these genes, followed by capillary electrophoresis on an automated DNA analyzer to estimate the sizes of amplicons and the numbers of tandem repeats. For each target, a number was given according to the number of tandem repeats, and a MIRU type with a 12-digital number, which is derived from 12 numbers of tandem repeats of five targets, is given for each isolate. Susceptibility tests were performed with the proportion method. We also used the VNTR-MIRU analysis to investigate the laboratory cross contamination by *M. tuberculosis*.

### Results

Among 493 isolates of *M. tuberculosis* from 203 patients, 54 and 106 types were obtained by VNTR and MIRU typing, respectively. When the results of VNTR and MIRU were combined, 125 VNTR-MIRU types were obtained. Among the genotypes, four VNTR-MIRU types were shared by eight to 32 nonrepetitive isolates, and the remaining 121 types were representative by a single isolate or were shared by isolates from two to four patients. One presumed false positive and four possible false positives were detected by the VNTR-MIRU analysis and clinical analyses.

### Conclusions and Suggestions

In this study, an automated MIRU method for typing *M. tuberculosis* has

been developed. The MIRU method has been shown to be more discriminatory than the VNTR method, and the combined use of MIRU and VNTR methods were found to be more discriminatory than the use of MIRU typing alone. The VNTR-MIRU method can be used not only for epidemiological studies on tuberculosis but also for investigation of laboratory cross-contaminations by *M. tuberculosis*. More studies are needed to investigate the links among the isolates showing the same genotypes, the most common genotype in particular.

**Keywords :** *Mycobacterium tuberculosis* ; Mycobacterial Interspersed Repetitive Units ; polymerase chain reaction ; database ; variable-number tandem repeats ; Spoligotyping