Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Microbiological Methods 65 (2006) 425–431
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Comparison between agarose gel electrophoresis and capillary electrophoresis for variable numbers of tandem repeat typing of *Mycobacterium tuberculosis*

Eiji Yokoyama [a,*], Kazunori Kishida [a], Masako Uchimura [a], Sadato Ichinohe [b]

[a] *Division of Bacteriology, Chiba Prefectural Institute of Public Health, 666-2 Nitona, Chuo, Chiba City, Chiba, 260-8715, Japan*
[b] *Division of Infectious Disease Epidemiology, Chiba Prefectural Institute of Public Health, 666-2 Nitona, Chuo, Chiba City, Chiba, 260-8715, Japan*

Received 12 July 2005; received in revised form 25 August 2005; accepted 31 August 2005
Available online 10 October 2005

Abstract

Variable numbers of tandem repeat (VNTR) typing of *Mycobacterium tuberculosis* was performed on 54 strains including 23 strains derived from 9 outbreaks. PCR amplicon sizes of 12 *mycobacterial* interspersed repetitive unit tandem repeat loci were measured using both agarose gel electrophoresis and capillary electrophoresis. Similarities using agarose gel electrophoresis of Euclidian distances among the 23 strains derived from the 9 outbreaks were significantly lower than that using capillary electrophoresis (Wilcoxon signed ranks test, $P<0.01$). By clustering analysis using unweighted pair group method using arithmetic averages, all of the 23 strains derived from the 9 outbreaks were each clustered with more than 90% similarities based on the distance using capillary electrophoresis. In contrast, differential clusters with more than 90% similarity were observed with only 7 strains derived from 3 outbreaks when analyzed by agarose gel electrophoresis. These results indicated that measurement of PCR amplicon size of tandem repeat loci should be carried out using capillary electrophoresis and that agarose gel electrophoresis is not suitable for clustering analysis of *M. tuberculosis* VNTR typing.

Keywords: *Mycobacterium tuberculosis*; Agarose gel electrophoresis; Capillary electrophoresis; Variable numbers of tandem repeat (VNTR); *Mycobacterial* interspersed repetitive unit (miru); Clustering analysis

1. Introduction

Tuberculosis caused by *Mycobacterium tuberculosis* remains one of the most important infectious diseases. The number of tuberculosis patients in Japan is still higher than that of other developed countries (Aoki, 2001). Tuberculosis has been increasing among homeless people in Japan (Aoki, 2001; Ano et al., 2002) and it is difficult to investigate individual contacts of such patients. Cluster analysis based on IS*6110*-restriction fragment length polymorphism (RFLP) of the organism has been used to trace transmission of the organism between the patients when epidemiologic relationships are unclear (Takahashi, 2003; Ano et al., 2002; Crawford et al., 2002; Barden et al., 2002). However, this analytical method has disadvantages of being both time-consuming and requiring large amounts of DNA. Despite a proposal for standardized methodology (van Embden et al., 1993), it remains difficult to compare

* Corresponding author. Tel.: +81 43 266 6723; fax: +81 43 265 5544.
E-mail address: e.ykym@ma.pref.chiba.jp (E. Yokoyama).

0167-7012/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2005.08.014

large numbers of RFLP patterns from different laboratories because of the lack of reproducibility (Kremer et al., 1999; Yang, 2003).

Recently, variable numbers of tandem repeat (VNTR) typing was used for fingerprinting of *M. tuberculosis* (Frothingham and O'Connell, 1998; Supply et al., 2000; Cowan et al., 2002; Le Flèche et al., 2002). VNTR typing is based on the polymorphic numbers of tandem repeats at various loci. PCR primers for VNTR typing were positioned at flanking regions of both sides of the tandem repeat locus. After amplification, the amplicon size is reduced by subtraction of the size of flanking regions. The size after subtraction of the amplicon is divided by the size of one repeat unit. The calculated value represents the number of repeat units at a locus.

Two methods have been used for measuring amplicon size. After agarose gel electrophoresis, amplicon size is measured using fingerprint-type analysis computer software (Balckwood et al., 2004) or by visual comparison to molecular weight markers (Mokrousov et al., 2004; Mazars et al., 2001; Frothingham and O'Connell, 1998; Cowan et al., 2002; Le Flèche et al., 2002). Agarose gel electrophoresis is inexpensive and simple, but distortions during electrophoresis may influence the measurements. Moreover, it is impossible to obtain accurate repeat numbers with decimal fractions of tandem repeat in visual inspection, even though it has been reported that the repeat numbers were sometimes not integers, but numbers with a decimal fraction (Le Flèche et al., 2002). The sequence analysis data from "The Microorganism Tandem Repeat Database" revealed that 9/10 VNTR loci of *mycobacterial* interspersed repetitive units (miru) in *M. tuberculosis* H37Rv and CDC1551 strains showed the repeat numbers with decimal fractions (http://minisatellites.u-psud.fr/GPMS/default GV.php), suggesting that the numbers of tandem repeat should be measured with decimal fractions. In contrast, capillary electrophoresis (Allix et al., 2004; Sun et al., 2004a) is a method that provides a high level of accuracy for VNTR typing. However, the size of the amplicon has been reported to vary according to the type of internal size marker used (Lindstedt et al., 2004a).

In this study, we investigated VNTR typing of *M. tuberculosis* strains, including some epidemiologically related strains that possessed highly similar RFLP patterns. We measured amplicon size of tandem repeat loci by both agarose gel electrophoresis and capillary electrophoresis. The clonal relatedness obtained by both methods was compared to that determined by the RFLP analysis.

2. Materials and methods

2.1. Bacterial strains

A total of 54 *M. tuberculosis* strains were used in this study and 23/54 strains were derived from 9 outbreaks (outbreaks A–I). All of the strains from a single outbreak showed more than 90% similarity in RFLP analysis (Table 1). When the strains from 9 different outbreaks were compared, similarities were under 90%.

31/54 strains were isolated from patients whose contacts were not known based on epidemiology. A total of 6 pairs (unknowns A, C, D, F, and G) and one group (unknown B) of epidemiologically unrelated strains showed more than 90% similarity in the RFLP analysis. Strains of type B showed 100% similarity with strains of outbreak C (Table 1).

2.2. RFLP analysis

All 54 strains were analyzed by RFLP using the IS*6110* probe (van Embden et al., 1993) modified as described previously (Kishida et al., 2005). Briefly, electrophoresis was done using 1.4% agarose gels with external molecular weight markers only. DNA molecular weight marker III DIG-labeled (Roche, Mannheim, Germany) was used as external marker and

Table 1
M. tuberculosis strains investigated in this study

Outbreak	Numbers of strains	Epidemiological contact	Similarity of RFLP patterns
A	2	Family	100%
B	6	Hospital	96.2–100%[a]
C	3	Sauna	100%
D	2	Hospital	100%
E	2	Family	100%
F	2	Office	100%
G	2	Old-age home	100%
H	2	Hospital	100%
I	2	Old-age home	100%
Unknown A	2	Unknown	100%
Unknown B	3	Unknown	100%[b]
Unknown C	2	Unknown	100%
Unknown D	2	Unknown	97.1%
Unknown E	2	Unknown	100%
Unknown F	2	Unknown	96.8%
Unknown G	2	Unknown	100%

[a] Three strains showed identical RFLP patterns. Two strains showed one band added to the RFLP pattern and the similarities between the original and one added band patterns were 97.2%. One strain showed 2 added bands in RFLP patterns and the similarities between the original and two added band patterns were 96.2%.

[b] All strains also showed 100% similarity between RFLP patterns of outbreak C derived strains.