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Spoligotyping Followed by Double-Repetitive-Element PCR as Rapid Alternative to IS6110 Fingerprinting for Epidemiological Studies of Tuberculosis

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A total of 129 clinical isolates of *Mycobacterium tuberculosis* representing 91 patients were typed by a combination of direct-repeat (DR)-based spoligotyping and an inter-IS6110-PGRS (polymorphic GC-rich region)-PCR, also designated double-repetitive-element PCR (DRE-PCR). During the first phase of this investigation, 72 clinical strains representing 52 patients were initially typed by IS6110-restriction fragment length polymorphism (RFLP) and DR-RFLP, followed by spoligotyping and DRE-PCR. In the second phase of this investigation, the discriminating ability of spoligotyping plus DRE-PCR was studied for 57 isolates from 39 patients who were suspected to be epidemiologically linked, and the typing results were later confirmed by IS6110-RFLP and DR-RFLP analyses. The molecular clustering of the isolates remained identical irrespective of the methods used. These results show that the association of two PCR-based fingerprinting techniques for molecular epidemiology of tuberculosis has a discriminating ability similar to the IS6110-RFLP reference method.

Molecular fingerprinting of *Mycobacterium tuberculosis* with the transposon IS6110 (12) is useful for epidemiological studies (10), and an internationally accepted restriction fragment length polymorphism (RFLP) procedure has been described previously (13). However, the development of rapid typing methods remains important, as IS6110-RFLP requires culturing, DNA extraction, and Southern hybridization, and may take as long as 4 to 5 weeks. Moreover, IS6110 fingerprinting may be limited, as some strains may not harbor a copy of IS6110 whereas others may contain only 1 to 5 copies (14). In this context, alternative PCR-based techniques seem particularly promising, as they may help with both rapid diagnosis and molecular typing of tuberculosis; however, the methods described may not be sufficiently discriminatory when used alone (2, 6, 8). For this reason, a combination of rapid methods with spoligotyping as a first-line test was recently hypothesized as a potential alternative to IS6110-RFLP (3). In this paper, we describe a combination of spoligotyping (6) followed by double-repetitive-element (DRE)-PCR (2) as a rapid alternative strategy for *M. tuberculosis* typing.

A total of 129 clinical isolates, representing 91 patients, that were isolated at the Pasteur Institute of Guadeloupe from 1994 to 1996 and identified as *M. tuberculosis* by classical mycobacteriology procedures were the subject of the present investigation. DNA was prepared by the CTAB (cetyltrimethylammonium bromide) method (13) for the IS6110-RFLP, direct-repeat (DR)-RFLP, and DRE-PCR procedures. For spoligotyping experiments, DNA was prepared by a microbead disruption method (1). IS6110-RFLP and DR-RFLP analyses were performed as reported previously (11, 13). Spoligotyping was performed with membranes that were prepared locally (6). DRE-PCR was performed as reported previously (2); a 20 25-μl aliquot of the PCR mixture was analyzed on 2% agarose gels, and pictures were taken after ethidium bromide staining by using a video-copy system and Gel-Analyst software (Bio-probe, Montreuil, France). IS6110-RFLP, DR-RFLP, and DRE-PCR results were analyzed with Taxotron software (Taxolab, Institut Pasteur, Paris, France), as reported previously (11). Spoligotyping results were entered in a spreadsheet (Excel) file and ordered for cluster identification.

TABLE 1. Molecular description of clusters observed for 72 *M. tuberculosis* clinical isolates from 52 patients by IS6110-RFLP and DR-RFLP analyses, followed by spoligotyping and DRE-PCR

Cluster	No. of IS6110-RFLP bands	No. of DR-RFLP bands (size [bp])	Spoligotype	No. of major DRE-PCR bands (size [bp])	No. of strains ^a
A	4–5	2 (4,800; 3,000)	■□□□□□□□□□□■□■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	1 (180)	12
B	13	1 (5,100)	■■■■■■□□□□□□□□□□□□□■□□□□□□□□□□□□□□□□■■■■■■	1 (625)	3
C	9–11	2 (4,750; 3,000)	■□■■■■■■■■■■■■■■■■■■■■□□□□□□□■□□□□■■■■■■■	1 (500)	2

^a The number of clustered isolates was based on IS6110-RFLP analysis followed by DR-RFLP analysis.

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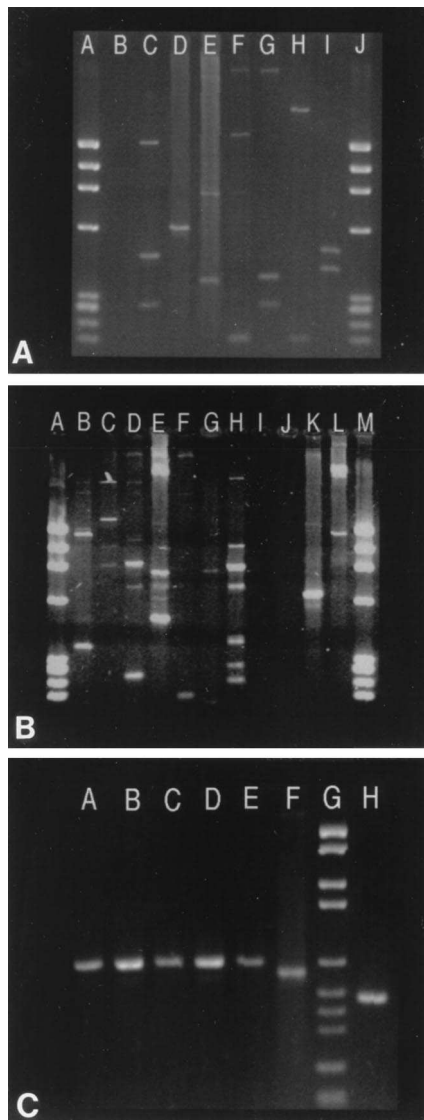


FIG. 1. Representative patterns obtained following DRE-PCR analysis of *M. tuberculosis* clinical isolates. (A and B) Results for isolates that were clustered by neither IS6110-RFLP nor spoligotyping. (A) Lanes: A, molecular weight (MW) marker; B, negative control; C to I, various unclustered isolates (GP14, GP35, GP16, GP5, GP25, GP33, and GP10); J, MW marker. (B) Lanes: A, MW marker; B to H, various unclustered isolates (GP1, PMON, GP34, GP9, A11, GP39, and GP15); I, negative control; J, an unclustered isolate, GP29, that did not generate any bands upon DRE-PCR; K, isolate B4 from cluster B; L, unclustered isolate GP37; M, MW marker. (C) DRE-PCR analysis of isolates that were clustered by IS6110-RFLP analysis followed by spoligotyping. Lanes: A to E, five isolates from four patients of cluster B; F, an unclustered isolate (GP20); G, MW marker; H, an isolate of cluster C. DNA MW markers IX (A and B) and VI (C), from Boehringer Mannheim, Meylan, France, were used.

patient isolates did not give visible bands upon DRE-PCR, which involved 4 unclustered isolates in this study). The use of DRE-PCR as a first-line test cannot be recommended, as it is not sufficiently discriminatory when used alone and both the

low (below 200 bp)- and high (above 3,500 bp)-molecular-size bands may be difficult to interpret and compare. Furthermore, interpretation of the results may be tedious when hundreds of isolates on separate gels are compared. In this sense, initial screening by spoligotyping limits the number of potentially linked isolates prior to DRE-PCR. In conclusion, the strategy described in this article is easily applicable to the handling of a very large number of samples and would be well suited to developing countries and/or countries with high prevalences of tuberculosis.

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