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An Improved Fluorescent Amplified Fragment Length Polymorphism Method for Typing *Mycobacterium tuberculosis*

We have evaluated fluorescent amplified fragment length polymorphism (FAFLP) fingerprinting as a complementary technique for genotyping *Mycobacterium tuberculosis*, which may aid in the elucidation of the transmission dynamics of tuberculosis. Earlier FAFLP studies (1, 2, 3, 5) broadly employed EcoRI and MseI restriction enzymes, which are known to have a low cleavage frequency for GC genomes of >65 mol% (4). By contrast, BamHI and MspI restriction endonucleases were used in this study because they have a higher cleavage frequency (as judged by in silico calculations) for the *M. tuberculosis* genome and do not show polymorphisms within IS*6110*/IS*986*.

Study 1. FAFLP was performed on 39 IS*6110* restriction fragment length polymorphism (RFLP) genotyped strains. The FAFLP profiles of these isolates generated an average of 74 amplified fragments between 50 bp and 400 bp, and 24 of these bands were polymorphic. Cluster analysis differentiated between two main groups. The first cluster consisted of seven strains with multiple copies of the IS*6110* element, and they lacked the 118-bp and 243-bp fragments but shared 201-bp and 286-bp polymorphic bands. The second cluster was comprised of strains with only a single copy of the IS*6110* elements, which were poorly differentiated by RFLP, and all possessed the 200-bp and 286-bp polymorphism bands. These single-copy strains were further grouped into four subgroups, differentiated by 229-bp, 243-bp, 288-bp, 316-bp, and 372-bp polymorphic fragments.

Study 2. Study 2 consisted of 44 *M. tuberculosis* strains, all of which were previously characterized by IS*6110* RFLP. Analysis of these FAFLP profiles produced two main clusters (Fig. 1). The banding patterns of the 10 isolates belonging to the first cluster correlated with those of the single IS*6110* copy isolates because of the presence of the 200-bp and 286-bp polymorphisms. However, these strains lacked the 243-bp polymorphism, which was prominent in the single IS*6110* copy strains of study 1. The second cluster consisted of multiple IS*6110* strains, and they were divided into three subgroups, as shown in Fig. 1.

Epidemiological and medical records of the source patients of these study 2 isolates revealed that the patients were residents of London, United Kingdom, and were of either Somalian or Caucasian ethnic origin. The IS*6110* RFLP data that were obtained for comparison purposes (6) show significant genetic heterogeneity within strains of both Caucasian and Somalian origins. A direct comparison of the FAFLP and the IS*6110* RFLP data for the 42 strains revealed that only isolates 99/1154 and 00/10121 of Caucasian origin clustered at 100% genetic similarity for the two typing techniques. However, visual inspections of the IS*6110* profiles indicate that samples of the same FAFLP cluster have significantly similar though not identical RFLP banding patterns. This suggests a significant degree of congruence between the two typing techniques (Fig. 1).

Is this FAFLP method dominated by the IS*6110*/IS*986* genetic marker? As there was some shared congruence between FAFLP and IS*6110* RFLP patterns, an obvious question that needed addressing was whether the FAFLP using BamH1 and MspI was reliant on IS*6110*, since these restriction enzymes have restriction sites within the IS*6110* element.

Although sequences flanking the IS*6110* elements can be detected as FAFLP, experiments utilizing selective nucleotides complementary to sequences immediately around BamHI and MspI sites within the IS*6110* element detected none of the observed polymorphisms (Fig. 2). This confirmed that BamHI plus T and MspI plus 0 FAFLP was not influenced by the IS*6110* element and the flanking sequences, which has important implications for the complementation of this approach to IS*6110* RFLP.

Conclusions. The differentiation of *M. tuberculosis* isolates containing only a single copy of IS*6110* confirms the potential of FAFLP as a highly discriminatory fingerprinting technique which is complementary to IS*6110* RFLP. Furthermore,

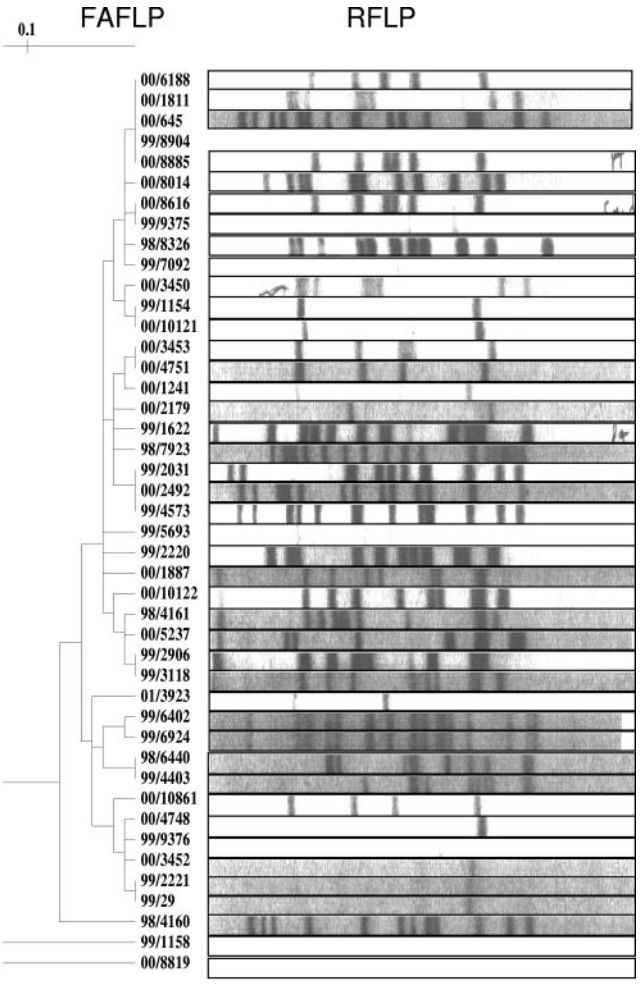

FIG. 1. Comparison of the FAFLP dendrogam and the IS*6110* RFLP patterns of all 44 isolates. Clustering employed the unpaired-group method with arithmetic averages by use of a multivariate statistical package.

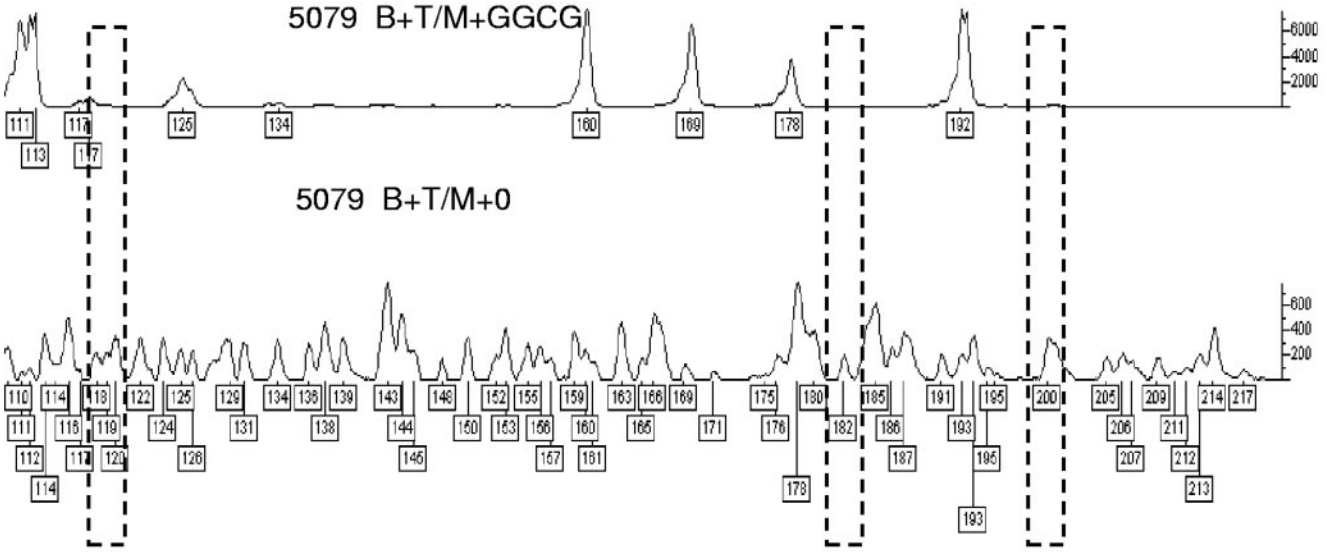

FIG. 2. FAFLP profiles from BamHI plus T (B+T)/MspI plus 0 (M+0) and BamHI plus T/MspI plus GGCG (M+GGCG) for *M. tuberculosis* isolate 5079, demonstrating that the observed polymorphisms (dashed boxes) are not generated by the IS*6110* element. The boxed numbers under the peaks are fragment sizes in base pairs, and the vertical scales at right are fragment intensities.

BamHI and MspI FAFLP requires only two primers, compared to eight primers for EcoRI and MseI multiplexing done by Goulding et al. (3), which has resource implications. Since only one data set was generated for each isolate using BamHI plus T and MspI plus 0 FAFLP, our approach involves less data analysis and as such we believe it will significantly enhance subsequent interlaboratory data comparison.

In conclusion, the results presented in this study strongly suggest that in conjunction with IS*6110* RFLP, FAFLP using BamHI plus T and MspI plus 0 could significantly improve strain identification of *M. tuberculosis* and in turn the control of tuberculosis.

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