**Application of FLiP Method for Differentiation of Mycobacterium tuberculosis Strains in Comparison to Commonly Used Methods, Spoligotyping and MIRU-VNTR Typing**

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Received 11 April 2012, revised 27 September 2012, accepted 28 September 2012

**Abstract**

The current "gold standard" in molecular epidemiological studies of Mycobacterium tuberculosis is IS6110 RFLP based on IS6110 polymorphism. However PCR-based methods are becoming increasingly important. Recently, fast ligation-mediated PCR (FLiP), based on IS6110 polymorphism was proposed. In this study, the discriminatory power of FLiP, spoligotyping and MIRU-VNTR typing, in differentiation of M. tuberculosis isolates was compared. The discriminatory index (HGI) of spoligotyping, MIRU-VNTR analysis, and FLiP was 0.653, 0.837, and 0.917, respectively. This indicates that FLiP allows a high level of differentiation among M. tuberculosis strains and it might be a valuable alternative to the other typing methods.

**Keywords:** Mycobacterium tuberculosis, FLiP; MIRU-VNTR typing, spoligotyping
Fig. 1. Genetic relationships among 25 isolates of M. tuberculosis based on spoligotyping (A), MIRU-VNTR (B) and FLiP (C) analyses. Clusters of identical patterns are indicated in curly brackets.
Sajduda et al., 2006; Allix-Béguec et al., 2008). Further extension of the set of loci to 24 sequences resulted in higher resolution power comparable to that of the reference method (Maes et al., 2008).

Fast ligation-mediated PCR (FLiP) is a novel method, based on IS6110 polymorphism. It involves digestion of chromosomal DNA by restriction enzyme HhaI and ligation with a synthetic oligonucleotide adaptor sequence (Reisig et al., 2005). Such product is then PCR amplified using primers complementary to the IS6110 and adaptor sequence, and resolved by polyacrylamide gel electrophoresis. The discriminatory power of FLiP was reported to be close to that of the IS6110 RFLP (Kremer et al., 2005; Reisig et al., 2005). However, very few analyses of M. tb. strains were performed using FLiP, and its comparison to other commonly used PCR-based methods is therefore required.

In this study, we used FLiP to estimate molecular relationships among 25 strains of M. tb., isolated from TB patients in 2006–2007. FLiP analysis was performed as originally described by Reisig et al., 2005. FLiP genotyping data were compared to the results previously obtained by 15-locus MIRU-VNTR typing and spoligotyping, two PCR-based methods commonly used in molecular epidemiology of TB (Krawczyk et al., 2011).

Spoligotyping grouped the analyzed strains into three types: S1, S2, and S3, consisting of 8, 12 and 5 strains, respectively (Fig. 1A.). The discriminatory power of spoligotyping, expressed as HGI (Hunter-Gaston index; Hunter and Gaston, 1988), was 0.653. The 15-locus MIRU-VNTR analysis divided the 25 strains into 8 classes: M1–M8 (Fig. 1B.). Within each of the types S1 and S2, the 15-locus MIRU-VNTR distinguished three classes: M6, M7, M8, and M1, M2, M4, respectively. Type S3 was subdivided into classes M3 and M5. The classes M2, M6 and M3 contained 8, 6 and 3 strains, respectively. The classes M1, M4, and M5 contained two strains each, whereas classes M7 and M8 were represented by single strains. The HGI for 15-locus MIRU-VNTR typing was 0.837. Combining it with spoligotyping increased HGI to 0.930 (data not shown).

The FLiP method subdivided 25 strains tested into 12 clusters, leading to further differentiation of strains belonging to classes M4 and M6 (Fig. 1C.). Class M4 was subdivided into single strains F10 and F11, and class M6 was split up into clusters F3 and F4. Class M2 was diversified into four clusters: F6, F7, F8, and F9 with the exception of strain no. 513. This strain was included into the cluster F4 together with strain no. 632, belonging to the class M8 in MIRU-VNTR typing. In comparison with the two other methods, FLiP showed the highest discriminatory potential (HGI = 0.917) in differentiation of the strains tested.

In conclusion, spoligotyping can be used as a screening method for molecular epidemiological investigations of large numbers of M. tb. strains. The high discriminatory power presented by FLiP suggests it as an interesting alternative method for the widely used MIRU-VNTR typing. However, in large numbers of strains tested, the use of MIRU-VNTR typing could be more appropriate, since it requires determination of the size of a single PCR product instead of comparison of patterns, as in FLiP. FLiP appears to be a relatively simple and fast method, reliably discriminating M. tb. strains. However, further studies of strains from worldwide distribution would be necessary to prove its wide applicability.

Acknowledgments
This work was supported by grant no. N N302 111338 from the National Science Centre, and partially performed within the project (POPW.01.03.00-18-018/09) “Center of Applied Biotechnology and Basic Sciences”, supported by the Operational Program “Development of Eastern Poland 2007–2013”.

Literature


