Proposal of a new method for subtyping of *Mycobacterium kansasii* based upon PCR-restriction enzyme analysis of the *tuf* gene

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Introduction and Aim. *Mycobacterium kansasii*, an important cause of pulmonary disease, closely resembling pulmonary tuberculosis, is one the most frequently isolated clinical species of non-tuberculous mycobacteria (NTM) (1). There are seven recognized *M. kansasii* subtypes or genotypes I-VII (2). This intraspecies heterogeneity has clinical and epidemiological implications. Types I and II are the most prevalent and have been associated with human disease, whereas the other five (III-VII) are predominantly of environmental origin (3, 4). Currently, there are two major methods used for the identification of *M. kansasii* subtypes I-VI, with either *hsp65* or *rpoB* gene coding for the 65-kDa heat shock protein and β-subunit of RNA polymerase, respectively, or 16S-23S rDNA internal transcribed spacer (ITS) as molecular targets (5-7). Recently, the *tuf* gene, coding for the Tu (thermo-unstable) elongation factor (EF-Tu) has been exploited as a useful marker for the taxonomic classification of mycobacteria (8). The aim of this study was to investigate whether the *tuf* gene can be used for subtyping of *M. kansasii*.

Materials and Methods. Six bacterial strains, representatives of six *M. kansasii* subtypes (I-VI) were used in the study. All strains were cultured on Löwenstein-Jensen medium. Genomic DNA was extracted with the cetyl-trimethyl-ammonium bromide (CTAB) method, as described elsewhere (9). For the amplification of the *tuf* gene fragment (*ca*. 740 bp) a PCR protocol with T1 and T2 primers was employed (8). The same primers were used for sequencing of the obtained amplicons. Based on the sequence data, the search for a restriction enzyme that would yield genotype-specific patterns and simulations of restriction digestions were performed *in silico* by using insilico.ehu.es software. The DNA fragments were electrophoresed and visualized by staining with ethidium bromide and UV fluorescence.

Results. Upon *in silico* analysis, MvaI was found as the only enzyme to produce distinct patterns for each of the six (I-VI) *M. kansasii* subtypes and thus was selected for PCR-restriction enzyme analysis (-REA) assay. The amplicons representing partial *tuf* gene sequence were MvaI-digested and the resulting DNA fragments matched exactly those established *in silico*. The specific and unique restriction patterns determined for *M. kansasii* subtypes I-VI are given below (**Table 1**).

Table 1. Differentiation of *Mycobacterium kansasii* subtypes by PCR-REA of the *tuf* gene with MvaI.

Subtype	Fragments length (bp)	
	designed *in silico*	expected on agarose gel
I	321, 87, 84, 70, 69, 59, 51	320, 90, 70, 60, 50
II	321, 121, 87, 84, 69, 58	320, 120, 90, 70, 60
III	321, 171, 71, 69, 58, 51	320, 170, 70, 60, 50
IV	492, 72, 63, 58, 51, 6	490, 70, 60, 50
V	390, 171, 71, 57, 51	390, 170, 70, 60, 50
VI	408, 120, 84, 72, 59	410, 120, 80, 70, 60

Conclusion. In this study, a new PCR-REA assay was proposed, which allows for differentiation of *M. kansasii* subtypes with the use of only one restriction enzyme. This could be a new molecular tool for the discrimination of *M. kansasii* subtypes.

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