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>
<thead>
<tr>
<th>Subtype</th>
<th>designed in silico</th>
<th>Fragments length (bp) expected on agarose gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>321, 87, 84, 70, 69, 59, 51</td>
<td>320, 90, 70, 60, 50</td>
</tr>
<tr>
<td>II</td>
<td>321, 121, 87, 84, 69, 58</td>
<td>320, 120, 90, 70, 60</td>
</tr>
<tr>
<td>III</td>
<td>321, 171, 71, 69, 58, 51</td>
<td>320, 170, 70, 60, 50</td>
</tr>
<tr>
<td>IV</td>
<td>492, 72, 63, 58, 51, 6</td>
<td>490, 70, 60, 50</td>
</tr>
<tr>
<td>V</td>
<td>390, 171, 71, 57, 51</td>
<td>390, 170, 70, 60, 50</td>
</tr>
<tr>
<td>VI</td>
<td>408, 120, 84, 72, 59</td>
<td>410, 120, 80, 70, 60</td>
</tr>
</tbody>
</table>
Conclusion. In this study, a new PCR-REA assay was proposed, which allows for differentiation of \textit{M. kansasii} subtypes with the use of only one restriction enzyme. This could be a new molecular tool for the discrimination of \textit{M. kansasii} subtypes.

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References