A new method for differentiation between members of the *Mycobacterium kansasii* complex

Katarzyna Rastawicka, Zofia Bakuła, and Tomasz Jagielski*

Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, I. Miecznikowa 1, 02-096 Warsaw, Poland

* Correspondence: Tomasz Jagielski, PhD, DSc; e-mail: t.jagielski@biol.uw.edu.pl

Background:

Only since recently, the hitherto existing subtypes (I-VI) of *Mycobacterium kansasii* have been elevated to species rank, based on whole-genome sequence analysis. *Mycobacterium kansasii* (former subtype I), *M. persicum* (II), *M. pseudokansasii* (III), *M. ostraviense* (IV), *M. innocens* (V) *and M. attenuatum* (VI), along with *Mycobacterium gastri* have been placed within the *M. kansasii* complex (MKC).
Currently, the most widely used approach allowing for MKC species identification involves, depending on the protocol, PCR amplification of partial *tuf*, *hsp65*, or *rpoB* genes, followed by restriction enzyme digestion of the amplicons. Importantly, all these assays are prone to inaccuracies, often misidentifying *M. kansasii* as *M. persicum.*
The aim of this study was to design a new, fast and simple, one-step PCR assay that would provide an accurate identification of each of the MKC species.

Material/methods:

The study included 158 *Mycobacterium* sp. genomes deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). This number comprised 67 genomes of all MKC species, 60 genomes of 12, other-than MKC, non-tuberculous species, and 31 genomes of the *Mycobacterium tuberculosis* complex species. The analyzed genomes were searched for sites that would yield easily detectable amplicons of different sizes among the MKC species, while producing no amplicons for other-than MKC *Mycobacterium* species. The primer pairs were mapped with Bowtie 2 against all *Mycobacterium* genomes, which was followed by filtering of the resulting files using in-house Python scripts. Each set of primers were tested *in vitro* as a mix of 4 primers in one reaction on the type strains of the MKC species.

Results:

Based on the assumed criteria, three sets of primer candidates were designed with computer assistance. Only one primer combination produced amplicons *in vitro* consistent, in number and size, with those expected upon *in silico* analysis. Thus, for each MKC species, a species-characteristic profile was produced.

Conclusions:

This study offers a new PCR-based method for an accurate identification of species belonging to the MKC. Unlike the previous protocols, our method was validated using type strains of all MKC species. It is a single-step protocol, with easily produced and interpretable results. A large-scale evaluation of the method is currently underway.

Key words: *Mycobacterium kansasii* complex, multiplex PCR, molecular typing, nontuberculous mycobacteria