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

Only since recently, the hitherto existing subtypes (I-VI) of *Mycobacterium kansasii* have been elevated to species rank, based on whole-genome sequence analysis. New species and closely related *M. gastri* have been placed within the *M. kansasii* complex (MKC) (**Table 1**). 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 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.

Figure 1. *M. kansasii* at Löwenstein–Jensen medium.

Table 1. Members of the MKC.

MKC species:	Former subtype
M. kansasii	I
M. persicum	II
M. pseudokansasii	III
M. ostraviense	IV
M. innocens	V
M. attenuatum	VI
M. gastri	-

Methods

The study included 158 *Mycobacterium* sp. genomes deposited in the GenBank database. This number included 67 genomes of all MKC species, 60 genomes of 12, other-than MKC, non-tuberculous species, and 31 genomes of 4 species of the *Mycobacterium tuberculosis* complex. 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 reference strains.

Results

Based on the assumed criteria, three sets of primer candidates were designed with computer assistance. Only one primer produced amplicons *in vitro* (**Figure 1**) consistent, in number and size, with those expected upon *in silico* analysis combination (**Table 2**). Thus, each MKC species produced a species-characteristic profile.

Figure 2. PCR patterns obtained using a newly designed method for different MKC species. Lanes: M - Size marker; I - *M. kansasii;* II - *M. persicum;* III - *M. pseudokansasii;* IV - *M. ostraviense;* V - *M. innocens;* VI - *M. attenuatum;* VII - *M. gastri.*

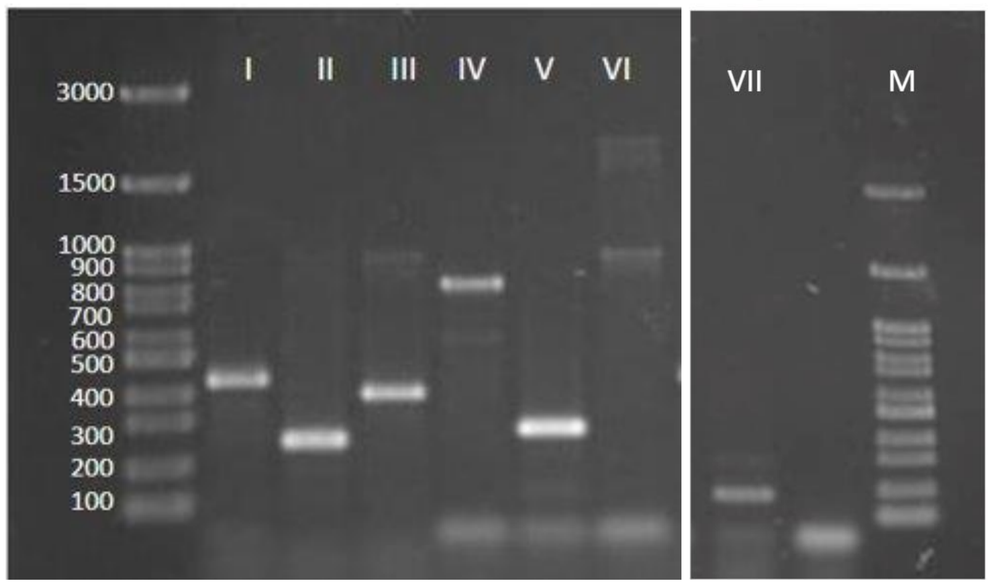

Table 2. Amplicons size for each of MKC species (bp) obtained using a newly designed primer set.

Species	I *M. kansasii*	II *M. persicum*	III *M. pseudokansasii*	IV *M. ostraviense*	V *M. innocens*	VI *M. attenuatum*	*M. gastri*
Product	450	260; 986	392; 985	830	287	999	188

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.